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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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VOLUME LI.

WOODS HOLE, MASS.

JULY TO DECEMBER, 1926

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BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY.

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I. TRUSTEES.

(AS OF AUGUST 11, 1925.)

EX OFFICIO.

FRANK R. LILLIE, *Director* and *President of the Corporation*, The University of Chicago.

MERKEL H. JACOBS, *Associate Director*, The University of Pennsylvania.

LAWRASON RIGGS, JR., *Treasurer*, 25 Broad Street, New York City.

GARY N. CALKINS, *Clerk of the Corporation*, and *Secretary of the Board of Trustees*, Columbia University.

EMERITUS.

CORNELIA M. CLAPP, Mount Holyoke College.

GILMAN A. DREW, Eagle Lake, Florida.

MARINE BI LIBRA MASS

TO SERVE UNTIL 1929.

C. R. Crane, New York City.
I. F. Lewis, University of Virginia.
R. S. Lillie, The University of Chicago.
E. P. Lyon, University of Minnesota.
C. E. McClung, University of Pennsylvania.
T. H. Morgan, Columbia University.
D. H. Tennent, Bryn Mawr College.
E. B. Wilson, Columbia University.

TO SERVE UNTIL 1928.

H. H. Donaldson, Wistar Institute of Anatomy and Biology.
W. E. Garrey, Tulane University.
Caswell Grave, Washington University.
M. J. Greenman, Wistar Institute of Anatomy and Biology.
R. A. Harper, Columbia University.
A. P. Mathews, The University of Cincinnati.
G. H. Parker, Harvard University.
C. R. Stockard, Cornell University Medical College.

TO SERVE UNTIL 1927.

H. C. Bumpus, Brown University.
H. E. Crampton, Barnard College, Columbia University.
W. C. Curtis, University of Missouri.
George T. Moore, Missouri Botanical Garden, St. Louis.
W. J. V. Osterhout, Member of the Rockefeller Institute for Medical Research.
J. R. Schramm, University of Pennsylvania.
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Lorande L. Woodruff, Yale University.

TO SERVE UNTIL 1926.

E. G. Conklin, Princeton University.
Otto C. Glaser, Amherst College.
Ross G. Harrison, Yale University.
H. S. Jennings, Johns Hopkins University.
F. P. Knowlton, Syracuse University.
M. M. Metcalf, Oberlin, Ohio.
William Patten, Dartmouth College.
W. B. Scott, Princeton University.

Executive Committee of the Board of Trustees.

Frank R. Lillie, *Ex. Off. Chairman.*
Merkel H. Jacobs, *Ex. Off.*
Lawrason Riggs, Jr., *Ex. Off.*
E. G. Conklin, to serve until 1926.
C. R. Stockard, to serve until 1926.
I. F. Lewis, to serve until 1927.
T. H. Morgan, to serve until 1927.

The Library Committee.

C. E. McClung, *Chairman.*
M. M. Metcalf.
J. R. Schramm.
E. E. Just.
Robert A. Budington.
Charles J. Fish.
A. H. Sturtevant.
Alfred C. Redfield.

II. ACT OF INCORPORATION.

No. 3170

Commonwealth of Massachusetts.

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, Henry B. Pierce, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand, Eight Hundred and Eighty-Eight.

[SEAL] HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY.

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The President of the Corporation, the Director and the Associate Director of the Laboratory, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of

Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER.

To the Trustees of the Marine Biological Laboratory.

Gentlemen: I beg to submit my report as treasurer for the year 1925.

The books have been audited by Messrs. Harvey S. Chase & Company, Certified Public Accountants. A copy of their report is on file at the Laboratory and is open to inspection of any member of the Corporation.

The principal of the Endowment Fund at the close of the year consisted of securities of the book value of $905,452.50 and cash

$454.50. The income from the Endowment Fund was $46,276.21 and the fee of the Trust Company as Trustee was $787.50, leaving a net income of $45,488.71, or a little over 5 per cent. on the total fund. A full list of the securities will be found in the Auditor's report.

The securities in the Lucretia Crocker Fund are the same as reported last year and of the book value of $4,011.17, and there is a balance in cash of $487.04.

The land, buildings, library and equipment of the Laboratory, excluding the Gansett and Devil's Lane property represents an investment of $1,234,537.85 and after deducting $79,931.87 for depreciation, a net book value of $1,154,605.98.

At the close of the year the indebtedness of the Laboratory consisted of $3,127.40 in accounts payable and $54,030.01 in mortgages on its real estate.

Following is the balance sheet at the end of the year and the condensed statement of income and outgo for the year.

Exhibit A.

Marine Biological Laboratory Balance-Sheet, December 31, 1925.

Assets.

Endowment Fund Assets:			
Securities in Hands of Trustee—Schedule I		$ 905,452.50	
Investment Cash in Hands of Trustee		454.50	
		$ 905,907.00	
Lucretia Crocker Fund Assets,			
Securities—Schedule II	$ 4,011.17		
Cash—Schedule II	487.04	4,498.21	$ 910,405.21
Plant Assets:			
Land—Schedule III		$ 112,088.14	
Buildings—Schedule III		953,646.40	
Equipment—Schedule III		168,803.31	
		$1,234,537.85	
Less Reserve for Depreciation		79,931.87	
		$1,154,605.98	
Cash in Building Fund		7,262.76	1,161,868.74

Current Assets:			
Cash,			
In New York Bank	$ 765.88		
In Hands of Trustee	2,200.00		
In Falmouth Bank	2,801.88		
Petty Cash	500.00	$ 6,267.76	
Accounts and Notes—Receivable		18,434.33	
Inventories,			
Supply Department	$ 26,269.50		
Biological Bulletin	4,870.27	31,139.77	
Investments,			
Devil's Lane Property	$ 30,406.96		
Gansett Property	5,038.02		
Stock in General Biological Supply House, Inc.	12,700.00		
Retirement Fund Assets	2,398.34	50,543.32	
Prepaid Insurance		3,971.37	111,356.55
			$2,182,630.50

Liabilities.

Endowment Funds:			
Friendship Fund, Inc.		$ 405,000.00	
John D. Rockefeller, Jr.		400,000.00	
Carnegie Corporation		100,000.00	
Gain on Sale of Securities		907.00	
		$ 905,907.00	
Lucretia Crocker Fund		4,498.21	$ 910,405.21
Plant Funds:			
Rockefeller Foundation	$500,000.00		
Friendship Fund Gift, 1925	221,608.61		
Other Investments in Plant from Gifts and from Current Funds	414,760.13		
Total—Schedule V		$1,136,368.74	
Real Estate Mortgages:			
Gilman A. Drew Property	$ 15,000.00		
Danchakoff Property	10,500.00	25,500.00	1,161,868.74
Current Liability and Surplus:			
Investment Land Mortgages,			
Devil's Lane Property	$ 25,000.00		
Gansett Property	4,030.01	$ 29,030.01	
Accounts-Payable,			
Sundry Accounts	$ 3,127.42		
Due to Retirement Fund	2,398.34	5,525.76	
Items in Suspense		172.90	
		$ 34,728.67	

Current Surplus,				
Balance, January 1, 1925......		$ 83,639.02		
Add: Balance of Income for Year—Exhibit B......		16,427.73		
Amount realized on old accounts written off as bad................		390.80		
		$100,457.55		
Deduct Transfer to "Plant Funds":				
Payments from Current Funds for Land, Buildings, and Equipment during Year as shown in Schedule V..	$23,141.45			
Payments from Current Funds on New Laboratory Building Schedule IV.	1,688.22	24,829.67	75,627.88	110,356.55
				$2,182,630.50

EXHIBIT B.

MARINE BIOLOGICAL LABORATORY, INCOME AND EXPENSE, FOR YEAR ENDED DECEMBER 31, 1925.

	Total.		Net.	
	Expense.	Income.	Expense.	Income.
Income, Endowment Fund......		$ 46,278.21		$46,278.21
Donation for Expenses:				
National Academy of Sciences		500.00		500.00
Instruction....................	$ 7,518.34	9,872.50		2,354.16
Research......................	3,077.40	9,575.00		6,497.60
Biological Bulletin and Membership Dues..................	5,463.45	6,529.49		1,066.04
Supply Department, Schedule VI.	54,090.73	57,771.67		3,680.94
Mess, Schedule VII......	28,290.98	30,092.55		1,801.57
Dormitories, Schedule VIII...	7,410.36	5,612.81	$ 1,797.55	
Interest and Depreciation charged to above Three Departments. See Schedules VI, VII, and VIII..................(red)	14,228.05			14,228.05

Dividend on Stock in General Biological Supply House, Inc.		1,270.00		1,270.00
Rent of Microscopes		359.50		359.50
Interest on Bank Deposits		219.06		219.06
Newman Cottage	76.75	150.00		73.25
Sundry Income		206.14		206.14
Maintenance of Plant:				
Maintenance of Buildings and Grounds	$ 8,939.10			
New Laboratory Expense	11,185.82			
Chemical Department Expense	5,424.54			
Library Department Expense	5,011.71			
Sundry Expense	1,436.68			
Carpenter Department Expense	1,333.81			
Truck Expense	949.44			
Bar Neck Property Expense	441.75			
Pumping Station	91.67			
Evening Lectures	125.44			
Janitor's House	8.34		34,948.30	
General Expenses:				
Administration Expense	$ 13,462.77			
Endowment Fund Trustee	787.50			
Interest on Loans	510.00			
Bad Debts	437.27		15,197.54	
Reserve for Depreciation	$ 10,163.40		10,163.40	
		$168,436.93		$78,534.52
	$152,009.20	152,009.20	$62,106.79	62,106.79
Balance of Income carried to Current Surplus—Exhibit A		$ 16,427.73		$16,427.73

Respectfully submitted,
LAWRASON RIGGS, JR.,
Treasurer.

V. REPORT OF THE LIBRARIAN.
DECEMBER 31, 1925.

During the year 1925, the library has been established in its new quarters. There is now adequate room to place the serials, books, and reprints, so that they are readily available. The serial sets, arranged alphabetically by title, begin on the top floor of the stack-rooms and extend down to the center of the middle or reading-room floor. Our reference books begin where the serials end, covering the north end of this same floor on a level with the reading-room. These are grouped under subjects, while the reprints, alphabetical by author in arrangement, are confined to the floor below. A brief directory is placed at the foot of the stairway opposite the elevator and just off the reading-room, and another on the large bulletin in the reading-room.

In the large reading-room, a current serial rack, having a capacity for six hundred and thirty different serials, now contains three hundred and seventy-eight that are being received in issues more frequent than yearly. This summer the investigators found a list of daily receipts of these serials on the reading-room bulletin. Besides this, the custom of placing the day's receipts all together on a convenient reading table for the afternoon and evening of the day of their arrival will be continued another year. Two tables are reserved for the use of students and near these is a book-case to hold the special reference reprints and volumes assigned by the instructors for reading connected with the courses. An Encyclopedia Brittanica, a new world atlas (1924 Bartholomew edition), and a Webster's dictionary, installed in the reading-room this year, have already proved their usefulness. All new current books remained on exhibition in the reading-room throughout the summer, while a card catalogue gave the titles of all new books placed on the reference stacks since June of the previous year.

Besides the establishment of these few useful customs, the summer's experience made clear and definite two very important necessities. First, there must be silence in the reading-room, and second, current serials, current books, and back serial volumes must stay in place for immediate reference. Every effort will be made by the staff to establish these conditions. That the reading-room should be quiet goes without saying, of course. But occasions do arise when a serial volume must be taken out. Special permission given personally by the Librarian will gladly be allowed when this condition occurs. The use of reprints for outside consultation will continue to be encouraged, also, as a means of controlling the taking out of serials. With the comfortable tables and capacious side shelves in the stack-rooms, besides the space for forty-four readers at the round tables in the reading-room, the habit of using the library for reading should gradually replace the one of scattering the library contents throughout the buildings, and the necessity for assured quiet in the library is a necessary corollary to this changed custom.

The budget for the years 1924–1925 (not including salaries) were combined for the reasons stated in the 1924 report. This budget totalled $15,366.86. The expenditure was mapped out in the report as follows: books, $1,300.00; current subscriptions, $3,000.00; binding, $2,000.00; supplies, $500.00; express, $200.00; and back sets, $8,000.00. This money was actually spent in an apportionment as follows: books, $2,000.00; current subscriptions, $3,300.00; binding, $2,151.58; supplies, $584.58; express, $150.00; and back sets, $7,160.70; total, $15,366.86.

The large overlap of $700.00 in the amount spent for books is accounted for in three ways. This part of our library, including many monographs, has been neglected, in order to keep up our subscriptions to serials, and a certain accumulation of books, such as dictionaries, and even catalogues, had to be purchased; a part of the items are really properly assignable to back sets since they are important monograph series; and thirdly, the main bulk of the issue of Abderhalden: Handbuch der Arbeitsmethoden has come during these two years. The overlap in subscriptions of $300.00 is due to the fact that we had $300.00 of our appropriation left and so paid our subscriptions ahead for 1925 to this amount. This is done advisedly, also, for many new German subscriptions have been taken on in 1925 so that, in order to continue, we shall need more than our usual $1,500.00 in 1926 for this purpose. The binding ran over the $2,000.00

apportioned to that item only slightly, as did also the $500.00 for supplies. This latter item was greatly increased by the many notices we had printed and mailed to secure reprints.

The library now contains 15,000 bound volumes and 25,000 reprints. Of the 1,790 volumes accessioned during 1925, 1,492 were serial volumes and 298 were books. Of the books, 82 were gifts. The books of current issue, *i.e.*, 1924–25 were 82 in number.

Of the 500 currently received serials now on our cards, showing receipts within the last two years, 175 are paid subscriptions, although we include in this number, 23 "carry-on" orders for monograph series, and those reference works whose issues are being spread out through a number of years.

The work in caring for the library has been greatly improved during the year 1925 and its organization really established. With an assistant in charge of the reprints and a secretary in charge of the business of orders and receipts, a clear program of procedure in these two important phases of the library activities is functioning.

Growth in the catalogue is essentially connected with the cataloguing of our reprints. Some 7,000 cards were added to the catalogue this year. The 12,000 reprints acquired this year will when completed make a perceptible increase in the size of the catalogue.

Mention must be made of the acquisition by the Laboratory of a large part of Dr. Whitman's personal library and manuscripts. Although it is impossible to make, at this time, any detailed statement of the material in this, that is specifically valuable to us, I may say that in serials, besides duplicates that we may sell, a number of important gaps in our own sets will be filled, some of which are now entirely out of print; and in reprints, there are probably 10,000, at least one-half of which are not duplicates of those which we already have; and of books and monographs, many that are of great value.

The new current subscriptions for 1925 not listed in the 1924 report are: *American Journal of Tropical Medicine; Anatomischer Bericht; Annales de Chimie; Annales de Physiologie et de Physicochemie Biologique; Archiv für experimentelle Zellforschung; Archiv* *für Rassen- und Gesellschafts-Biologie; Archiv für wissenschaftliche Botanik (Zeitschrift für wissenschaftliche Biologie, Abt. E.); Archivio Italiano di Anatomia e di Embriologia; Berichte der deutschen chemischen Gesellschaft; Bibliographia Genetica; Bulletin of the American Association of University Professors; Centralblatt für allgemeine Pathologie und pathologische Anatomie; Comptes Rendus hebdomadaires des Séances de l'Académie des Sciences; Entomological News; Index Medicus; Journal of the Franklin Institute; Die Naturwissenschaften; Physikalische Zeitschrift; Proceedings of the Royal Society of London, Series A; Psyche; Quarterly Journal of Medicine; Resumptio Genetica; Revue générale de Botanique; Transactions of the Faraday Society; Vorträge und Aufsätze über Entwicklungsmechanik du Organismen; Zeitschrift für die gesamte experimentelle Medizin; Zeitschrift für Morphologie und Anthropologie; Zeitschrift für Morphologie und Oekologie der Tiere (Zeitschrift für wissenschaftliche Biologie, Abt. A.); Zeitschrift für Physik; Zeitschrift für wissenschaftliche Photographie, Photophysik und Photochemie;* and *Zell-Stimulationsforschung;* in all, thirty-two. *Entomological News*, the *Journal of the Franklin Institute* and *Psyche* are gifts. Dr. Harlow Shapley is presenting the current issues of the *Entomological News* and *Psyche;* and Dr. Samuel Pond presents the *Journal of the Franklin Institute* promptly as he finishes with the current issues.

New exchanges for the year 1925 not listed in the 1924 report are as follows: *Anales de la Sociedad Española de Fisica y Quimica; Annales de Biologie Lacustre; Archives internationales de Médecine expérimentale; Archivio di Scienze Biologiche; Beiträge zur Physiologie; Bericht der Senckenbergischen Naturforschenden Gesellschaft; Biologia Generalis; Bulletin de l'Institut des Recherches Biologiques; Bulletin de la Société de Chimie Biologique; Bulletin international de l'Académie Polonaise des Sciences et des Lettres, Cracovie; Bulletin of the Hydrological Institute of Russia; Japan Medical World; Journal de Chimie Physique; Journal of the Linnean Society: Botany; Klinische Wochenschrift; Mededeelingen van den Burgerlijken Geneeskundigen Dienst in Nederlandsch-Indie; Memoirs, Bulletins,* etc., *of the Boston Society of Natural History; Münchener Medizinischer Wochenschrift; Prace*

Zoologiczne; Publication of the African Association for the Advancement of Science; Reports of the Ichthyological Laboratory of Astrakan; Rivista di Biologia; Sprawozdania Stacji Hydrobiologicznej na Wigrach; Travaux de l'Institut Nencki; Travaux de la Société des Naturalistes de Petrograd; Travaux de la Station biologique de Roscoff; Wiener Klinische Wochenschrift; Zeitschrift für Tierzüchtung und Züchtungsbiologie; Zeitschrift für vergleichende Physiologie (Zeitschrift für wissenschaftliche Biologie, Abt. C.); and *Zentralblatt für Zoologie, allgemeine und experimentelle Biologie.* We were able to secure the first volume of the *Archiv für experimentelle Zellforschung* through the kindness of Dr. Paul Reznikoff who sent his copy of the *Journal of the American Medical Association* to Dr. Erdmann through the year 1925, in exchange for it. We shall have to subscribe to this in the future.

We have acquired the following back sets by purchase since January 1, 1925, expending for these $2,940.90: *Anatomische Hefte*, volumes 1–57; *Annales de l'Institut Pasteur*, volume 7; *Archiv für mikroskopische Anatomie*, volume 32; *Archiv für Rassen- und Gesellschafts-Biologie*, volumes 1–14; *Archivio di Fisiologia*, volumes 1–16; *Archives de Morphologie générale et expérimentale*, volumes 1–21; *Berichte der deutschen botanischen Gesellschaft*, volumes 1–22; *Bulletin de l'Institut Pasteur*, volumes 2, 3, 4, 6, and 7; *Bulletin of the United States Fish Commission*, volume 15; *Comptes Rendus de l'Academie des Sciences*, volumes 178–179; *Congrès Internationale de Zoologie*, 1893 and 1896; *Ergebnisse und Fortschritte du Zoologie*, volumes 1–5; *Jahresbericht über die Fortschritte der Anatomie und Entwicklungsgeschichte*, volumes 1–11, 13, and 20; *Journal de Chimie Physique*, volumes 1–21; *Journal of Metabolic Research*, volumes 1 and 2; *Journal of Pathology and of Bacteriology*, volumes 1–27; *Journal of Physiology*, volume 8; *Kolloidchemische Beihefte*, volumes 1–20; *Medical Science*, volumes 3–11 completed; *Norwegian North Atlantic Expedition*, 1876–1878, seven volumes; *Philosophical Transactions, Series B.*, volumes 178–212; *Quarterly Journal of Experimental Physiology*, volumes 1–11; *Vorträge und Aufsätze über Entwicklungsmechanik du Organismen*, volumes 1–20; *Zeitschrift für Biologie*, volumes 1–69; *Zeitschrift für biologische*

Technik und Methoden, volumes 1–3; *Zoologisches Zentralblatt*, volumes 1–18; *Zoologische Berichte*, volumes 1–4; and *Zoologisches Jahresbericht*, 1913.

The back sets secured by exchange have been very few and are as follows: *Beiträge zur Physiologie*, volumes 1–2; *Bulletin international de l'Académie Polonaise des Sciences et des Lettres*, 1912–1913; and *Medical Science*, volumes 1–11.

Through the kindness of publishers and authors, as well as those interested in the library, the following books have been received this year as gifts:

From the publisher, Blakiston: Bowden, Garfield A.: *General Science with Experimental and Project Studies;* Stitt, E. R.: *Practical Bacteriology, Blood Work and Animal Parasitology;* from the publisher, Ginn: Brown, William H.: *A Textbook of General Botany;* Gruenberg, Benjamin C.: *Biology and Human Life;* and *An Introduction to the Science of Life;* Pratt, Henry Sherring: *A Course in Vertebrate Zoölogy;* from the publisher Holt: Herrick, Glenn W.: *Manual of Injurious Insects;* Locy, William A.: *The Growth of Biology;* McEwen, Robert S.: *Vertebrate Embryology;* from the publisher, McGraw-Hill: Palmer, C. I.: *Practical Calculus for Home Study;* Steinmetz, Charles P.: *Four Lectures on Relativity and Space;* Wieman, H. L. (at the request of the author): *General Zoölogy;* from the publisher, Macmillan: Bailey, L. H.: *Manual of Cultivated Plants;* Baumgartner, W. J.: *Laboratory Manual of the Foetal Pig;* Bigelow, Robert Payne: *Directions for the Dissection of the Cat;* (at the author's request) Hunt, Harrison R.: *A Laboratory Manual of the Anatomy of the Rat;* Peabody, J. E. and Hunt, A. E.: *Biology and Human Welfare;* Trafton, Gilbert H.: *Biology of Home and Community;* from the publisher, Mosby: Macleod, John J. R.: *Physiology and Biochemistry in Modern Medicine;* from the publisher, Saunders: Dorland, W. A. N.: *American Illustrated Medical Dictionary;* Garrison, Fielding, H.: *An Introduction to the History of Medicine;* MacCallum, William G.: *A Textbook of Pathology;* Mallory and Wright: *Pathological Technique;* McClendon, J. F. and Medes, Grace: *Physical Chemistry in Biology and Medicine;* Morse, W.: *Applied Biochemistry* (at the author's request); Pearl, Raymond: *Intro-*

duction to Medical Biometry and Statistics; Sollmann, Torald: *A Laboratory Guide in Pharmacology;* and *A Manual of Pharmacology;* from the University of Wisconsin Press: *First Colloid Symposium Monograph;* from the publisher, Wiley: Curtis, C. Winterton and Guthrie, Mary: *Laboratory Directions in General Zoölogy;* from the publisher, Wood: Mathew, A. P.: *Physiological Chemistry* (at the author's request); from the Yale University Press (at the suggestion of Dr. L. L. Woodruff): Baitsell, George A., Editor: *The Evolution of Man;* Barrell, Joseph: *Evolution of the Earth and Its Inhabitants;* Thomson, J. Arthur: *Concerning Evolution;* from the author, Barrett, James W.: *A Vision of the Possible; The Australian Army Medical Corps in Egypt;* The Twin Ideals, *An Educated Commonwealth; The War Work of the Y. M. C. A. in Egypt; The Diary of an Australian Soldier; Save Australia;* from the author, Cannon, W. B.: *Bodily Changes in Fear, Hunger, and Rage;* from the author, Chumley, James: *The Fauna of the Clyde Sea Area;* from Colton, H. S.: Gould, Augustus A. and Binney: *Invertebrate of Massachusetts;* from the author, Cullen, Thomas S.: *Umbilicus; Cancer of the Uterus; Myomata of the Uterus;* and *Adenomyome of the Uterus;* from the author, Donaldson, H. H.: *The Rat,* 1924 edition; from Dr. Dunn: Meehan: *Flowers and Ferns of the United States;* from the author, Gage, Simon Henry: *The Microscope;* from the author, Jennings, H. S.: *Prometheus;* from Mrs. F. R. Lillie: Méheut, M.: *Etude de la Mer;* from Mrs. T. H. Montgomery, Jr.: Jennings, H. S.: *Behavior of the Lower Organisms;* from Mrs. A. McGee: Morse, Edward S.: *First Book of Zoölogy;* from the New York Library Board of the United Engineering Society: *Catalogue of Technical Periodicals in the Libraries of the City of New York and Vicinity;* from the author, Osborn, Henry Fairfield: *The Earth Speaks to Bryan;* from the author, Patten, Bradley M.: *Early Embryology of the Chick;* from Prât, S.: Nemec, B.: *Uvod do Vseobecne Biologie, Anatomie Rostlin;* and *Fysiologie Rostlin; L'Université Charles IV dans le Passé et dans le Présent;* from the author, Pratt, Henry S.: *A Course in Vertebrate Zoölogy;* from Tyndale, H. H.: *Oxford Dictionary;* from the author, Schaeffer, David N.: *The Millenium and Medical Science;* from the author, Scott, George G.: *The Science of Biology;* from the author, Smith, Gilbert M.: five copies of *Phytoplankton of the Inland Lakes of Wisconsin, part II, Desmidiaceæ;* from Miss Alice W. Wilcox: House: *Wild Flowers of New York* (plates); from the author, Wilson, Edmund B.: *The Cell in Development and Heredity;* from the author, Wolback, S. B.: *The Etiology and Pathology of Typhus;* and from Woodruff, L. L.: Ward, Henshaw: *Evolution for John Doe.*

Priscilla B. Montgomery,
Librarian.

VI. THE REPORT OF THE DIRECTOR.

To the Trustees of the Marine Biological Laboratory.

Gentlemen: I beg to submit herewith a report of the thirty-eighth session of the Marine Biological Laboratory for the year 1925.

1. *The New Building.*—The year 1925 was marked by the dedication and occupancy of the new building, planned for since 1919, as related in successive Annual Reports. A "plot" plan and floor plans of this building were published in the Annual Report for 1923 (Biological Bulletin, Vol. 47, 1924, pp. 29–35) together with a brief description of the uses of the rooms. The photographs herewith reproduced show a view of the completed building from the harbor, a closer view of the main facade, a view of the rear from the "Eel Pond," and interiors of a private biological and a private biochemical laboratory. The building is beautiful externally, and thoroughly practical and complete, as well as beautiful, in its internal arrangement and appointments. Its use during the season of 1925 revealed no serious flaws or defects in either its arrangement or its appointments.

The style of architecture is the same as that of the Crane Building, but accentuated by the use of stone for the basement walls, by horizontal stone courses above the first and third stories and by the use of painted copper panels between the windows of the second and third stories which increases the columnar

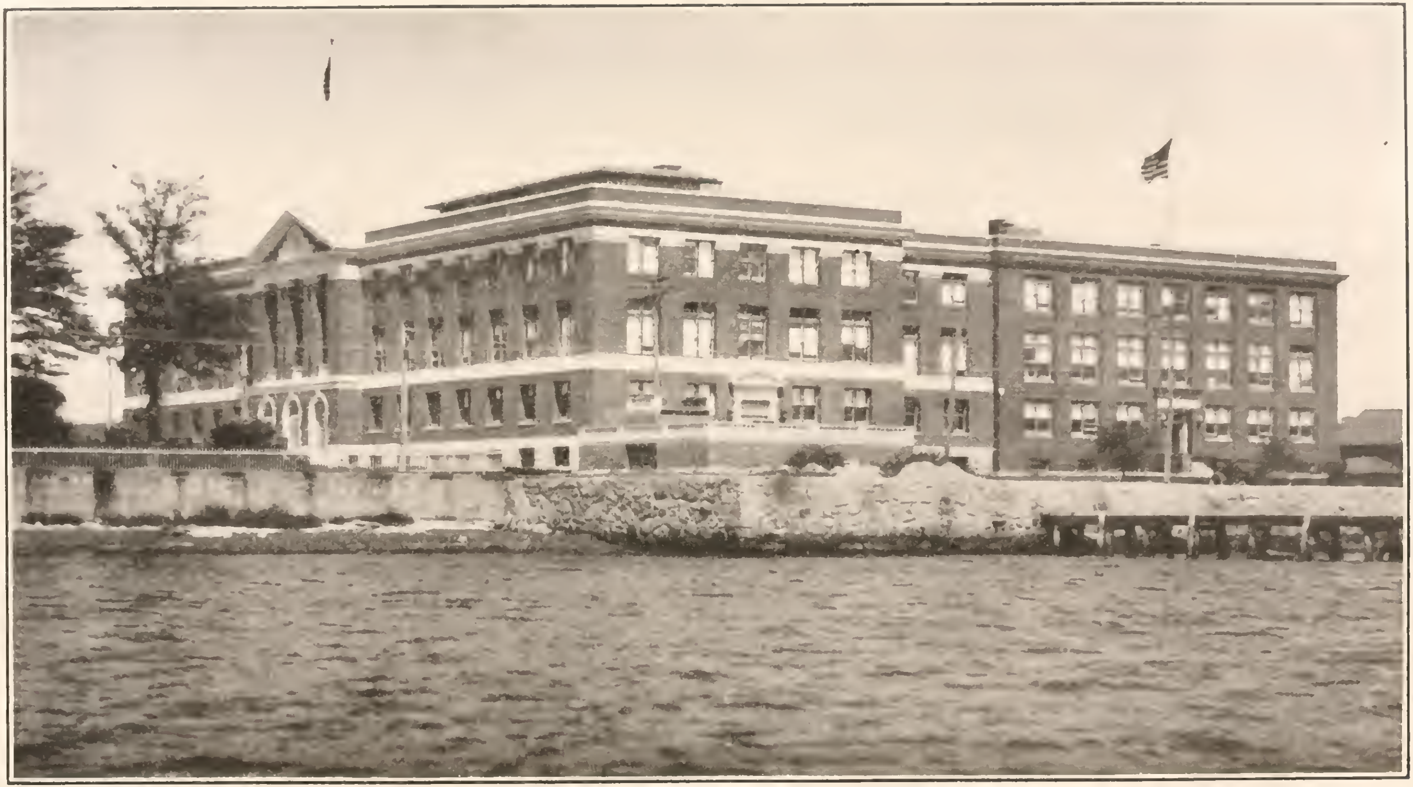

FIG. 1. The New Building and Crane Building (on the right) from Great Harbor. Note the three-quarters buried power house in the center at the corner of the stone harbor wall, and the roof of the tank house above the corner of the New Building.

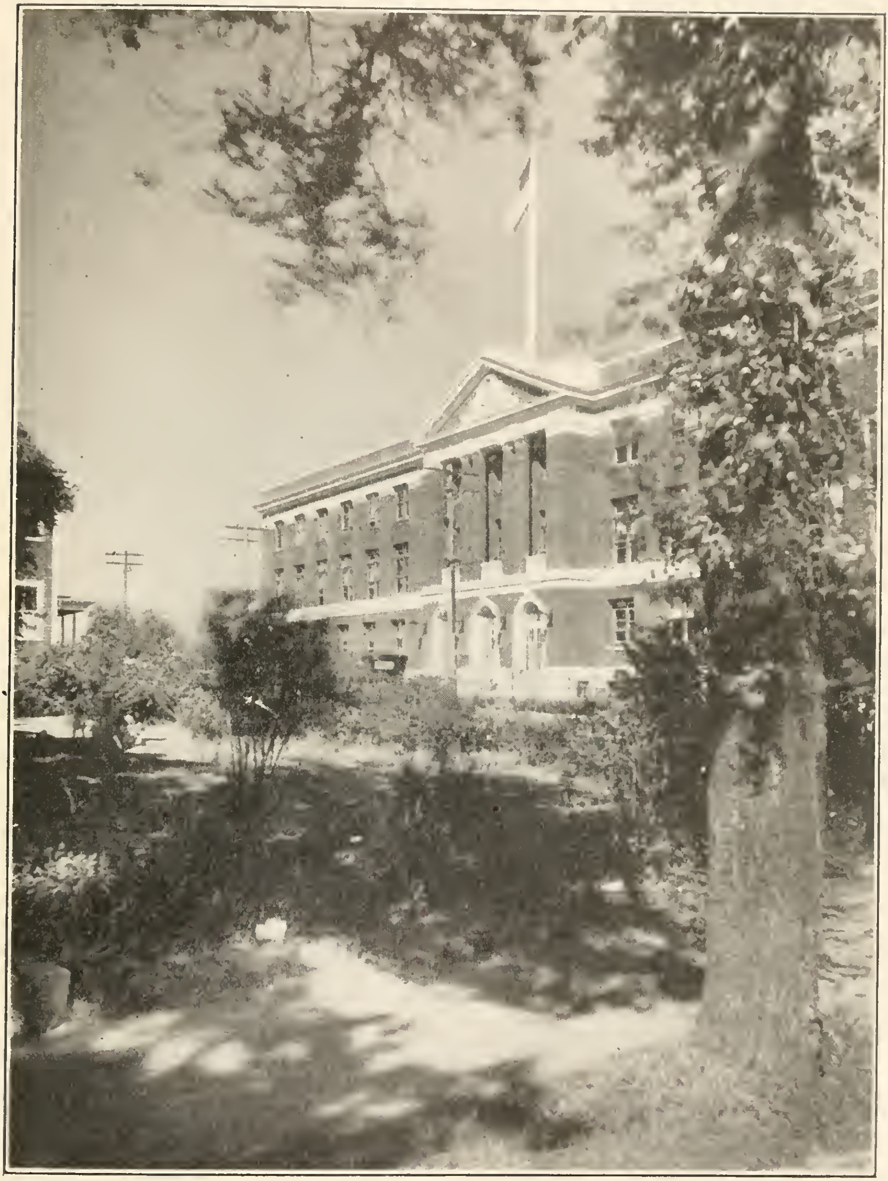

FIG. 2. A closer view of the main facade.

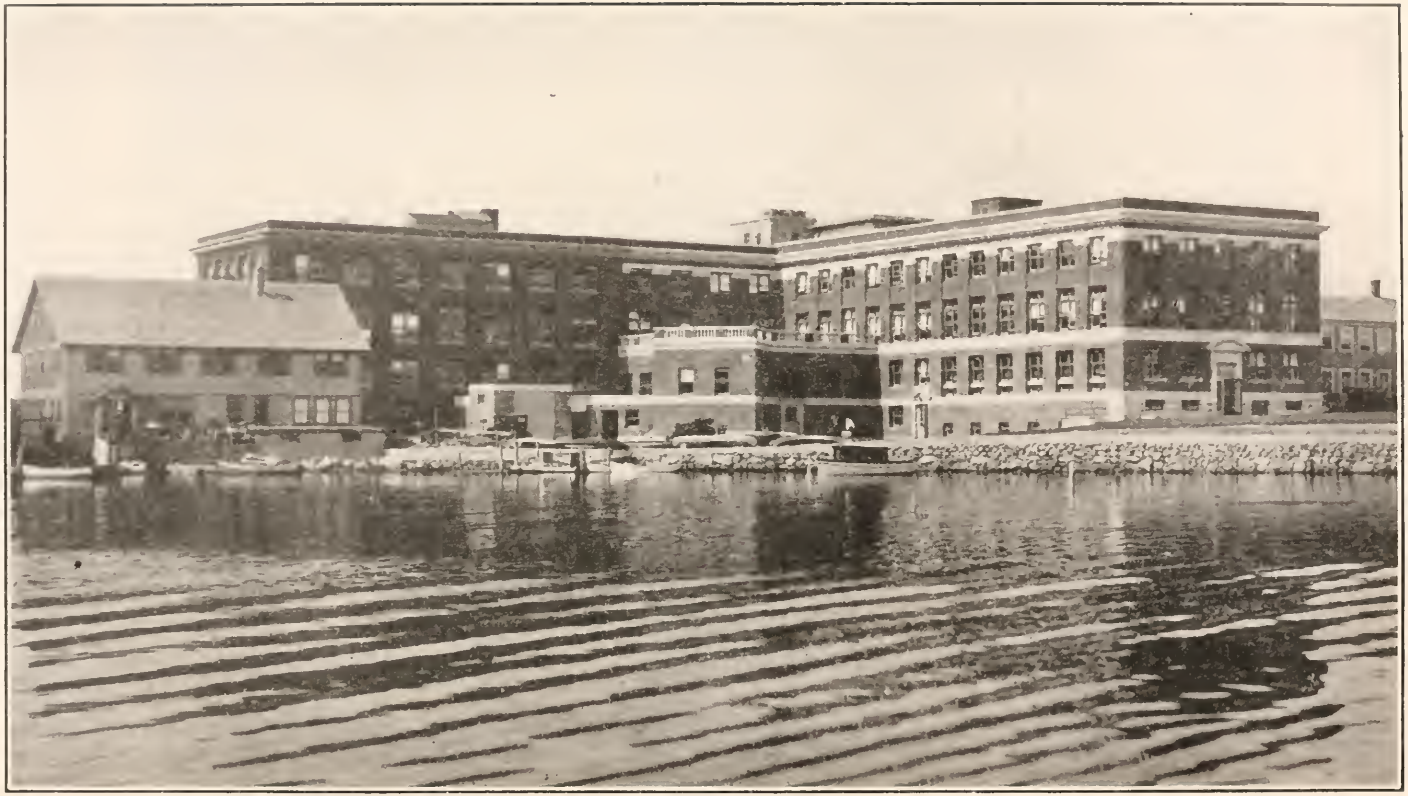

FIG. 3. Rear view of the New Building and the Crane Building (left wing) from the Eel Pond; wooden building of the Supply Department on the left. The auditorium projects from the interior angle.

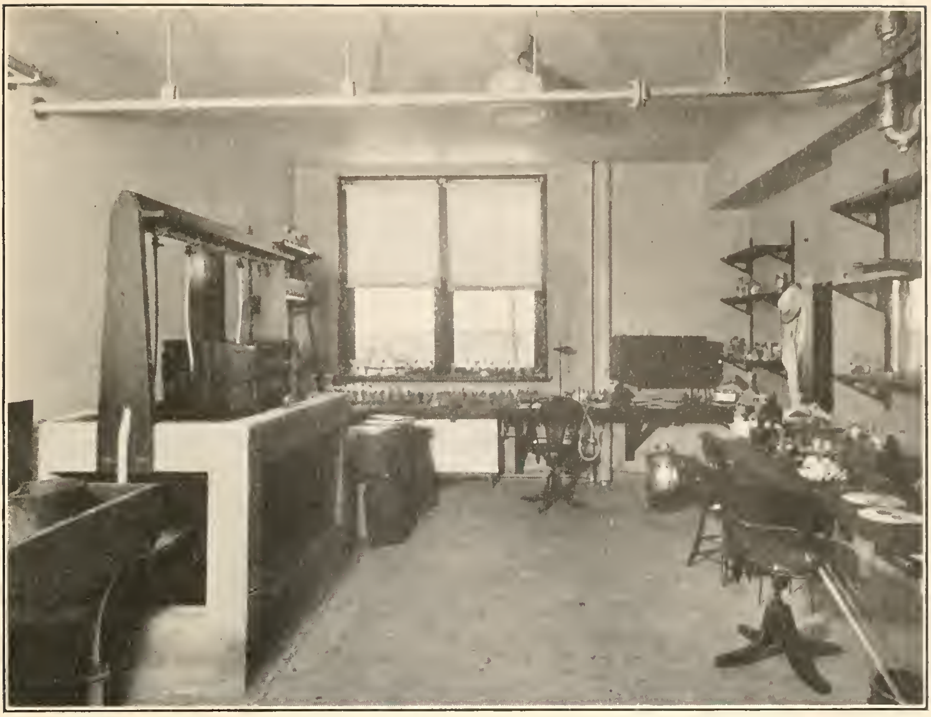

FIG. 4. Interior of one of the private biological laboratories overlooking Great Harbor and Nonamessett Island. Cement salt water table on left and usual furnishings, but special apparatus (14 x 20 ft.).

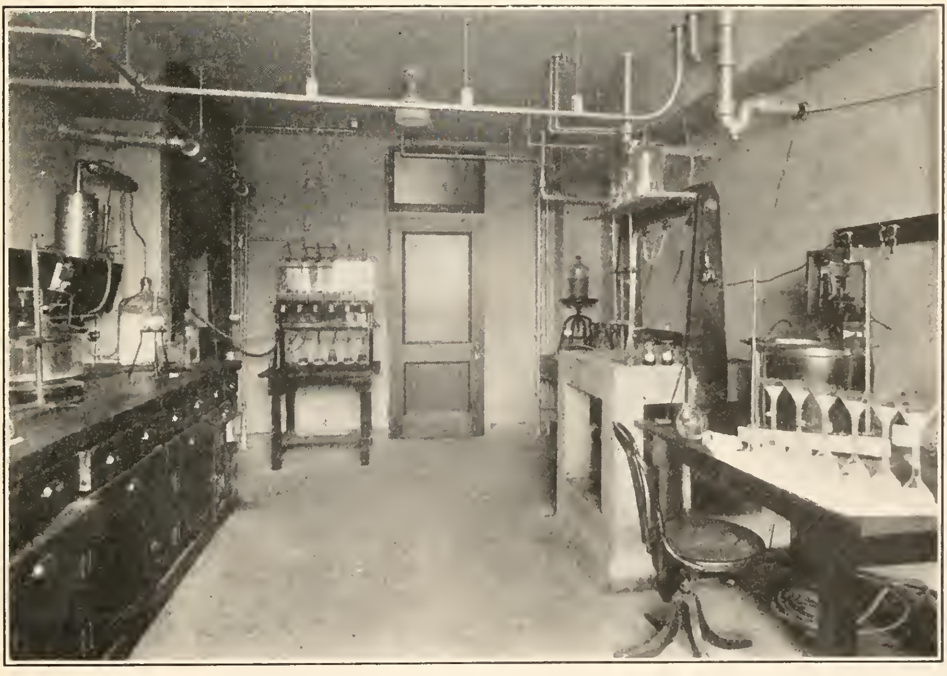

FIG. 5. Interior of one of the private biochemical laboratories taken from the window end. Note chemical desk and hood in addition to the fittings of a biological laboratory (12 x 23 ft.).

effects of the intervening brickwork. The main façade (Fig. 2) with its three high arched door-ways in the center, its rounded brick columns, and pediment ornamented with an intricate original marine composition are especially noteworthy.

2. The Dedication Exercises were held July 3, 1925. They were attended by official delegates of the following former and present subscribing and coöperating Institutions and Organizations, as well as by members of the Laboratory and neighbors.

OFFICIAL DELEGATES.

The Academy of Natural Sciences of Philadelphia	Professor E. G. Conklin Professor C. E. McClung
Agnes Scott College	Miss Mary Stuart MacDougall
The Department of Agriculture, Washington, D. C.	Dr. E. D. Ball
University of Alabama	President George H. Denny
The American Association of Anatomists	Professor Henry McE. Knower
American Association for the Advancement of Science	Dr. J. McKeen Cattell
The American Chemical Society	Dr. H. E. Howe
The American Society of Biological Chemists	Dr. Albert P. Matthews
The American Society of Naturalists	Professor S. O. Mast
The American Society of Zoölogists	Professor G. H. Parker
Amherst College	Professor Harold H. Plough
Antioch College	Mr. Ondess L. Inman
Barnard College	Professor Henry E. Crampton
Bermuda Biological Station for Research	Professor W. J. V. Osterhout
The Biological Laboratory, Cold Spring Harbor, L. I.	Dr. Reginald G. Harris
Boston Society of Natural History	Dr. Joseph A. Cushman
Boston University	Rev. Zerna Vane Arthur
Botanical Society of America	Professor B. M. Duggar Professor J. R. Schramm
Bowdoin College	Professor Manton Copeland
Boyce Thompson Institute for Plant Research	Dr. William Crocker

Bryn Mawr College	Dr. Franz Schrader
Bureau of Fisheries, Washington, D. C.	Dr. Willis H. Rich
Butler College	Dr. David Rioch
Carleton College	Professor D. B. Young
Carnegie Corporation	Professor T. H. Morgan
Carnegie Institution of Washington	Dr. A. F. Blakeslee
Carnegie Institution, Station for Experimental Evolution	Dr. E. C. MacDowell
The University of Chicago	Professor Ralph S. Lillie
University of Cincinnati	Dr. Edward F. Malone
Clark University	Dr. William H. Cole
The College of the City of New York	Assistant Professor Earl A. Martin
Columbia University	Professor Gary N. Calkins
	Professor Henry E. Crampton
	Professor Thomas H. Morgan
	Professor Edmund B. Wilson
Constantinople Woman's College	Dr. Mary Mills Patrick
Cornell University	Professor Charles R. Stockhard
University of Delaware, Women's College	Miss Margaret Walton
DePauw University	Professor Walter Norton Hess
Doane College	Mrs. M. C. Bennett
Elmira College	Miss Clara Dettmer
	Miss Elizabeth Humeston
University of Georgia	Dr. E. R. Clark
Goucher College	Professor Clara L. Bacon
Hamilton College	Professor A. D. Morrill
Harvard University	Professor W. J. V. Osterhout
	Professor George H. Parker
Harvard University Medical School	Dr. A. C. Redfield
Haverford College	Mr. William A. Wolff
Hood College	Dr. Mabel Bishop
Hope College	Professor Samuel O. Mast
Howard University	President J. Stanley Durkee
	Dr. E. E. Just
The Johns Hopkins University and the Johns Hopkins Medical School	Dr. Warren H. Lewis

Eli Lilly and Company	Dr. G. H. A. Clowes
McGill University	Dr. I. Maclaren Thompson
Marietta College	Professor H. R. Eggleston
University of Maryland	Professor P. W. Zimmerman
Massachusetts Institute of Technology	Professor Robert T. Bigelow
Mercer University	Professor Gail L. Carver
Miami University, Oxford, Ohio	Dr. J. K. Breitenbecker
University of Michigan	Professor L. V. Heilbrunn
James Millikan University	Dr. H. P. Agersborg
Milwaukee-Downer College	Dr. Mary Edith Pinney
University of Minnesota	Dean Elias P. Lyon
The Missouri Botanical Garden	Dr. Benjamin M. Duggar
University of Missouri	Dr. Charles H. Philpott
The Mount Desert Island Biological Laboratory	Dr. Ulric Dahlgren
Mount Holyoke College	Dr. Cornelia Clapp
National Research Council	Dr. Maynard M. Metcalf
The National Academy of Sciences	Dr. Frank R. Lillie
The New York Academy of Sciences	Professor Henry E. Crampton
The University of North Carolina	Dr. C. Dale Beers
Oberlin College	Professor Robert A. Budington
	Professor Charles G. Rogers
Ohio Wesleyan University	Professor E. G. Conklin
Peabody Museum, Salem, Mass.	Professor Edward S. Morse
University of Pennsylvania	Professor Clarence Erwin McClung
University of Pennsylvania School of Medicine	Professor Henry Cuthbert Bazett
Pomona College	Dr. Charles W. Metz
Randolph-Macon College	President R. E. Blackwell
Rhode Island State College	Dr. H. W. Browning
	Dr. F. Bauer
Rutgers College	Dr. Walter T. Marvin
Sheffield Scientific School, Yale University	Professor Ross G. Harrison
Shorter College	Dr. Ada R. Hall
Smithsonian Institution	Dr. Austin H. Clark
Stanford University	Mr. Charles Maurice Cram

St. Louis University	Professor Alphonse M. Schwitalla
Syracuse University	Professor C. W. Hargitt
The Tulane University of Louisiana	Dr. Walter E. Garrey
Union College	Professor James Watt Mavor
Vassar College	Professor A. L. Treadwell
University of Vermont	Dr. E. G. Spaulding
University of Virginia	Dr. Ivey Foreman Lewis
University of Washington	Mr. Louis G. Seagrave
Washington University	Mr. Alfred M. Lucas
Washington and Lee University	Professor W. D. Hoyt
Wesleyan University	Professor Hubert B. Goodrich
The Western College for Women	Dr. Ruth Laura Phillips
Western Reserve University and Adelbert College	Professor Francis H. Herrick
Whitman College	Rt. Rev. Robert L. Paddock
Williams College	Professor James L. Kellogg
The University of Wisconsin	Professor Charles E. Allen Professor Leon J. Cole
The Wistar Institute of Anatomy and Biology	Dr. Milton J. Greenman
Yale University	Professor Lorande L. Woodruff

Members of the Laboratory and many invited guests were also present and the large new auditorium was completely filled. The afternoon addresses according to the subjoined program have been published in *Science* (Vol. LXII., No. 1604, pages 271–280, Sept. 25, 1925). The Committee in Charge of the Exercises, under the able direction of Dr. H. E. Crampton, provided lunch and housing arrangements for delegates, and guides for inspection of the building, and arranged an afternoon tea and demonstrations of materials and experiments set up by workers at the Laboratory. The exercises were concluded by an evening lecture delivered by Professor W. J. V. Osterhout of Harvard University.

MARINE BIOLOGICAL LABORATORY.

WOODS HOLE, MASSACHUSETTS.

Dedication Exercises.

FRIDAY, JULY 3.
1925.

2 P.M. Daylight saving time. In the auditorium. Presiding Officer, The Honorable Charles R. Crane, *President Board of Trustees.*

Address of Welcome: Professor Frank R. Lillie, *Director.*

Address: Professor Edmund B. Wilson, Columbia University.

Address: "The Changing Face of Nature and of Man at Woods Hole," by Professor Edwin G. Conklin, Princeton University.

4:00–6:00 P.M. Inspection of buildings and work. Afternoon tea will be served.

8:00 P.M. The first of the series of evening lectures will be delivered by Professor W. J. V. Osterhout, of Harvard University—Subject: "Absorption and Accumulation."

3. *Report of the Building Committee* (The Director, Assistant Director and Treasurer).—In the last Annual Report the estimates for construction of the new building and the sources of funds for the purposes were given. It is a source of gratification to the Committee that the building was completed and equipped for a sum of $734,101.01 which is $14,485.70 within the estimates of $748,886.71. Ground was broken March, 20 1924 and the building practically fully equipped was ready for occupancy in June, 1925. In its final report presented to the Board of Trustees August 11, 1925 The Building Committee presented a summary of payments needed after July 1, 1925 as follows:

Summary of Additional New Building Costs after July 1, 1925.

July 1, 1925—	Balance due Contractors and Architects	$19,023.39	
	Balance due John Manville Co. for panel work in auditorium	1,500.00	
	Balance due on Supplementary Orders	22,321.70	$42,845.09

Additional Amounts Needed.

a. Apparatus and Chemicals	$ 5,000.00	
b. To complete Machine Shop	600.00	
c. Additional items for completion of service and equipment	5,000.00	10,600.00
		$53,445.09
July 1, Cash in Building Fund		30,094.48
		$23,350.61
Anticipated savings on contracts:		
Cleghorn Company	$817.00	
Cronin Company	925.00	1,742.00
August 3, Amount required from Friendship Fund		$21,608.61

In this accounting a fund of $10,600.00 was provided for minor items of completion. With this understanding the Committee proposed that their accounts be closed after the payment of the outstanding items noted above and that the Committee be discharged as soon as this is accomplished. The report was accepted by the Board of Trustees, and the Secretary was instructed to notify the Friendship Fund that the Marine Biological Laboratory would consider their pledge fulfilled by a total payment of $221,608.61 instead of $248,886.71 as originally estimated.

The subjoined analysis of the Building Fund Account was submitted by the Business Manager, Dec. 15, 1925.

ANALYSIS,
MARINE BIOLOGICAL LABORATORY,
BUILDING FUND ACCOUNT,
DECEMBER 15, 1925.

Cash Received to date:			
Rockefeller Foundation		$500,000.00	
Friendship Fund		221,608.61	
Interest on bank deposits:			
December, 1924	$ 8,721.67		
June, 1925	459.28	9,180.95	
Donation, Frank R. Lillie		3,311.45	$734,101.01
Payments as follows:			
Geo. A. Fuller Co., Gen. Contractor	$484,210.56		
C. H. Cronin, Inc., Plumbing	48,229.06		
Cleghorn Company, Heating	30,658.95		
Hixon Electric Co., Elec. work	66,555.36		
F. S. Payne Co., Elevator	3,977.00	$633,630.93	

Coolidge & Shattuck, Architects' commission and travelling exp.			31,734.26	
Other Commissions:				
Supt. and Eng. Fees:				
Heating—Buerkel & Co.	$ 518.94			
Elec.—Hixon Electric Co.	776.24	$ 1,295.18		
Elec.—Hixon Electric Co. (By Dr. Lillie, 1923.)	$ 1,990.51			
Heating—Buerkel & Co. (By Dr. Lillie, 1923.)	1,320.94	3,311.45	4,606.63	
Supplementary Items:				
	Paid.	*To complete.*		
Apparatus	$21,429.17	$ 2,070.83	$ 23,500.00	
Chemicals	1,474.84	25.16	1,500.00	
Crane Bldg. changes	6,205.16		6,205.16	
Furnishings	12,475.20		12,475.20	
Grading	1,889.75		1,889.75	
Machine Shop	3,999.72	500.28	4,500.00	
Sea water equipment	5,979.97		5,979.97	
Miscellaneous	1,844.75	4,734.36	6,579.11	
Johns-Manville, Inc. Auditorium ceiling	1,426.56	73.44	1,500.00	
	$56,725.12		$ 64,129.19	$ 64,129.19
Total Building Cost				$734,101.01
Balance on hand in Building Fund, Dec. 15, 1925		$ 7,404.07		

4. *Special Scientific Equipment of the New Building.*—Twenty-five thousand dollars was provided in the estimates for special scientific equipment, mostly movable apparatus, in addition to the apparatus already in possession of the Laboratory. The inventory value of the movable apparatus accordingly becomes well over $30,000.00. There has been provided thus such a quantity and variety of instruments and other apparatus, much of it very delicate and costly, that a new service department of "Scientific Apparatus" has been created with Dr. Samuel E. Pond of the University of Pennsylvana as custodian. Dr. Pond also acted as purchasing agent for the new apparatus, and was able to get the maximum results from the expenditure of the new

funds. There were few demands for apparatus in the season of 1925 that could not be satisfied with that on hand; the machine shop operated in coöperation with the department of scientific apparatus in adapting instruments and making new ones. All appliances are fully catalogued and cross-referenced as to distribution in the various buildings and rooms and temporary loans.

The equipment of the machine shop was completed during the summer. This equipment is largely for metal work. It is proposed to install a bench and equipment with a trained operator for glass blowing which will permit some construction of glass apparatus and all necessary repairs. It is not intended to centralize all glass blowing here, but to provide for the more delicate and complicated pieces of work.

5. *Attendance.*—Reference is made to the list of Students and Investigators in attendance (pp. 43–51) and to the Tabular View of Attendance (p. 51). The number of students admitted was held to the usual quota, which necessitated refusing a considerable number of applicants; the laboratories in the old buildings were used for the work of the classes as before. The number of investigators was 208 which is 14 more than the previous year the largest attendance heretofore. This season the space was ample. The policy in assigning space was determined partly by the rates, but, for the rest, the better equipped and more commodious quarters of the New Building and the Crane Building were filled first both for the beginning investigators and the older ones. All of the space in the brick buildings was thus occupied, and it was remarkable how little unoccupied space was left even in the old buildings after the need for crowding ceased and each investigator was given ample quarters. The number of institutions represented by students was 65, by investigators 74, combined 112 with allowance for duplications.

The following foreign countries and institutions were represented: Brazil, H. Paul's Medical School; China, Peking University and Shanghai; Czecho-Slovakia, Charles University Prag; England, University College, London; Germany, Kaiser Wilhelm Institut Für Biologie; Holland, University of Leyden and de Hoogere Burgerschool, Amsterdam; Poland, Lemberg and Warsaw Universities, Roumania, Bucharest University; Russia, Moscow University; Sweden, Lund University. The majority of the representatives of these Institutions were Fellows of the General Education Board, The International Education Board and the Rockefeller Foundation.

6. *The Report of the Treasurer* is printed on pp. 5–9. It includes the balance sheet as of December 31, 1925 and a condensed statement of expense and income for the year. These statements are taken from a comprehensive audit of the accounts of the Laboratory made, as in many previous years, by Harvey S. Chase and Company, Certified Public Accountants of Boston. The complete audit is on file at the Laboratory and may be examined by any member. The Treasurer has not included in his report the list of securities in the hands of the Trustee as they have not altered greatly from the list published last year. The total assets of the Laboratory have increased from $1,887,744.14 at the end of 1924 to $2,182,630.50 at the end of 1925. This increase is due for the most part to contributions from the Friendship Fund for completion of the new building amounting to $221,608.61, and to purchases of land, buildings and equipment made from current funds of the Laboratory amounting to $33,304.85. No part of the increase is accounted for by valuing up land or other property, but on the contrary a considerable sum has been charged off for depreciation of buildings and equipment.

The expense of operating the Laboratory increased from $125,118.91 in 1924 to $152,009.20 in 1925; and the income increased from $149,023.43 to $168,436.93 in the same years. The increase of expense is of course due in general to the cost of increased personnel and supplies needed for operation of the enlarged plant. The major part of the increase of income is distributed between the various departments, but a considerable share is due to increase of income from the Endowment Funds owing to the fact that they were established in 1924 and did not yield income for the first part of that year.

It is essential that the Laboratory should maintain a surplus in current accounts for improvements of a permanent nature, which are constantly needed in various departments of the Laboratory, and which it is impossible to secure in any other way. Such improvements are as necessary a part of the upkeep of a developing institution as the expendable supplies. The "Balance of Income carried to Current Surplus" has been expended, or invested in such ways, mostly on real estate; and liabilities on future "Current Surplus" have also been acquired mostly in the form of mortgages on newly acquired properties such as the Drew property, the Danchakoff property and Devil's Lane Tract. These mortgages must be discharged in the course of time, and some at least of future current surplus must be used for this account. Another charge on such funds consists of the annual payment of 5 per cent. of the payroll into the newly established retirement fund. Certain badly needed improvements in various departments of the Laboratory are also outstanding for which our current surplus has so far proved inadequate. Members should therefore not receive the impression that the surplus shown in the condensed statement of income and expense in the Treasurer's report represents money that could be used for luxuries.

7. *The Library.*—During the year Mrs. Montgomery was promoted to be Librarian. Her report (pp. 10–17) gives the actual progress for the year. The bound volumes on the shelves have increased from about 13,000 to about 15,000 and the reprints similarly placed on the shelves from about 10,000 to about 25,000 during the year. The Laboratory has now seriously undertaken the task of bringing the library service up to the level of the other services of the Laboratory to the scholar, an undertaking forecast by the splendid quarters provided in the new building and referred to in past reports. The Library Committee has accordingly in the last two or three years coöperated with the Librarian in a study of needs, and preparation of a report on the subject. This was made the basis of an appeal to the General Education Board for $50,000.00 to be expended as a capital sum in a five year period to complete the foundations of a really comprehensive research library in biology. This amount was appropriated by the Members and Trustees of the General Education Board at their meeting held November 19, 1925, and was accepted with hearty appreciation and thanks by the Executive Committee acting for the Laboratory. Together with

the regular annual appropriation of $6,750.00 a sum of $16,750.00 annually will be available for the next five years for accessions to the library and probably a slight addition to cost of administration. These accessions added to present possessions should complete the foundations of a comprehensive research library in Biology.

8. *Real Estate and Housing Conditions.*—These problems have received considerable attention during the last year. The future of the Laboratory could not be regarded as secure until sufficient land for future extensions was secured. The supply of lots in the Gansett property is approaching exhaustion; and the demand for real estate on the Cape is sending the price of land up very rapidly. Under these circumstances the Executive Committee decided to take advantage of an offer of the Fay Estate to sell a tract of land of about 105 acres, known as Devil's Lane tract, situated between the main Falmouth Road and the railroad, on very advantageous terms. This beautifully wooded tract is about one and a half miles from the Laboratory and it has the privileges to a right of way and a bathing reservation on the shore of Vineyard Sound. Part of the tract amounting to about 15 acres is good agricultural land, but most of it is hilly and well adapted to residence purposes. It is proposed to subdivide part for building purposes when the Gansett tract is sold out, restricting the sites to those connected with the Laboratory as in the case of the Gansett tract, and reserving the remainder for whatever purposes the Laboratory may serve in the future. During the year the Laboratory also purchased Dr. Drew's house and grounds, and repurchased Dr. Danchakoff's two lots and houses in the Gansett tract.

These purchases are in line with the policy of controlling sufficient real estate to be independent of artificial prices and rise in values. They do not, however, solve the housing problem. The Laboratory now maintains seven rooming houses and dormitories with accomodations for 172 persons at prices ranging from $2.00 to $4.00 per week. This is by no means sufficient in the height of the season; moreover some of the houses are old, and all lack good sanitary conveniences and rooms for social purposes. The largest of the houses will probably soon have to be abandoned. Village rentals either for separate rooms or for furnished houses tend to be unreasonably high. A very serious problem for the future of the Laboratory is that of the young married investigators especially if they have children. The Laboratory has no real provisions for family housing, and village rents are beyond the means of many of the most valued members of our organization. As a consequence in many cases they feel obliged to remain away from the Laboratory, which needs them, and which they need.

This situation is being studied and plans are being prepared to provide for an apartment house and a modern dormitory building in the immediate neighborhood of the Laboratory and a few small bungalows to be placed on the Gansett tract. At present funds are not available for such a considerable undertaking, but it is doubtful if they could be secured without a well considered plan. We shall accordingly proceed hopefully until the problem is solved.

9. *Changes in Personnel.*—Coincident with the establishment of the Endowment Fund and the completion of the New Building the past year has witnessed very important changes in personnel.

1. Resignation of Mr. C. R. Crane as President of the Corporation.

A. COPY OF MR. CRANE'S LETTER. TRUSTEES MINUTES, 1925, pp. 5–6.

August 8, 1925.

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY, WOODS HOLE, MASS.

Gentlemen: Twenty-two years have now elapsed since I became President of the Marine Biological Laboratory. I have enjoyed with you watching the growth of the Laboratory during that period. With the strong interest and support that is now assured, I feel that my own work has been completed and I hereby tender you my resignation which I ask you to accept.

The Laboratory has one unique feature in that its Board of Trustees consists almost entirely of biologists and it seems to me desirable that your President should also belong to the Fraternity. I have talked informally with some of the members of the Committee on Future Administrative Organization over the plan which they will propose at your meeting and I heartily approve the plan.

The future progress and prosperity of the Laboratory will always be a matter of great interest to me quite as much as if I continued to be your President.

Sincerely yours,
(signed) CHARLES R. CRANE.

B. LETTER OF ACCEPTANCE.

August 21, 1925.

HONORABLE CHARLES R. CRANE,
Woods Hole, Massachusetts.

Dear Mr. Crane: In regretfully accepting your resignation as President of the Corporation of the Marine Biological Laboratory the Board of Trustees appointed the undersigned members of the Board a Committee to express to you their grateful appreciation of the invaluable services which you have rendered to the Laboratory. You have been a member of our Board since 1901 and President of the Corporation since 1905 and throughout this period you have been our constant supporter and friend. During your Presidency you have seen the Laboratory grow, from a relatively small beginning, to the largest and most complete biological laboratory in the world, with assets totaling more than two million dollars.

Every year since your connection with our Board you have contributed most generously to the support of the Laboratory and for nearly twenty years you have lifted from our shoulders the burden of a large deficit. Only those who were members of the Board before your advent can fully appreciate the relief from financial worries and fears of suspension which your support has brought to us. Almost every year of your Presidency you have made notable additions to our estate, among which are the Kideer lot, cottage and annex, the Whitman and Ritter cottages, and New Homestead and Mess Hall, the Bar Neck Property, the Gansett Property, our first permanent and fireproof laboratory which should be known as the Crane Building, and finally the completion of the fund for the building and equipment of the New Laboratory and the permanent endowment of your annual gift of twenty thousand dollars from the Friendship Fund.

We are well aware that your interest in and support of the Laboratory has been a powerful factor in enlisting the coöperation of other benefactors and of great Foundations. If we have to-day one of the finest biological institutions in the world we owe it in large part to your faith and foresight.

Best of all has been the spirit of the support of yourself and Mrs.

Crane. Both of you have given generously and without any suggestion of conditions or interference. "The gift without the giver is bare," but you have given yourselves. Your spirit of helpful, friendly coöperation is the very spirit of this Laboratory which you have helped to create and confirm.

We regret that you are to be our President no longer, but we rejoice to know that you will still be a member of our Board of Trustees and an ever cherished colleague and friend.

Sincerely and cordially yours,
(signed) E. G. CONKLIN,
T. H. MORGAN,
EDMUND B. WILSON,
Committee.

To this record there is nothing to add. The Director was elected President of the Corporation by the Board of Trustees in Mr. Crane's stead.

2. Resignation of Dr. Gilman A. Drew as Assistant Director.

A. COPY OF DR. DREW'S LETTER OF DECEMBER 10, 1924.

"TO THE BOARD OF TRUSTEES OF THE
MARINE BIOLOGICAL LABORATORY.

Gentlemen: Unfortunately I have found it necessary to quit Woods Hole before the new building is completed.

This is a fitting time for me to sever my connection with the Laboratory and I herewith tender my resignation as Assistant Director, as Vice-President, and as a member of all Committees on which I am serving.

My membership on the Board is ex-officio and is automatically taken care of. Please do not elect me to fill a possible vacancy. I will not be able to attend meetings, and without direct connection my advice would rapidly fall in value.

I wish to thank you all for your never-failing support and to assure you that the continued welfare of the Laboratory will ever give me pleasure.

Sincerely yours,
(signed) GILMAN A. DREW."

In reply to this letter the Executive Committee granted Dr. Drew leave of absence until the next meeting of the Board, August 11, 1925, and at this meeting Dr. Drew's resignation was accepted as of December 31, 1925. In accepting the resignation of Dr. Drew, the Board of Trustees elected him Trustee *emeritus* and passed the following resolution which was ordered suitably engrossed on velum to be transmitted to Dr. Drew:

> The Board of Trustees of the Marine Biological Laboratory hereby expresses to Gilman Arthur Drew their great regret that he has found it necessary to resign as Assistant Director and their high appreciation of the invaluable services which he has rendered to the Laboratory as Instructor in Zoölogy and in Embryology from 1901 to 1916 and as Resident Assistant Director from 1910 to 1925.
>
> The evidences of his Scientific, Technical and Business ability are manifest everywhere in our Buildings, Equipment and Organization and his Zeal, Resourcefulness and Coöperation have helped to form the Spirit of this Institution.
>
> We extend to Dr. and Mrs. Drew our grateful thanks for all that they have done for the Marine Biological Laboratory and our best wishes for their future Health and Happiness.

Dr. Drew's resignation had been contemplated by him for some years on account of continued ill-health, but his devotion to the Laboratory was such that he risked, and finally incurred, serious illness by remaining at his post until the more essential features of the construction of the new laboratory were completed. The resolution expresses all too briefly the extent to which the Laboratory as an organization and a fully equipped plant is indebted to the long and devoted services of Dr. Drew. May his life on his citrus ranch in Florida to which he has retired bring him and Mrs. Drew good health and happiness!

3. Professor Merkel H. Jacobs of the University of Pennsylvania was elected by the Trustees on August 11, 1925 to succeed Dr. Drew and was given the title of Associate Director. For the present Dr. Jacobs will reside in Woods Hole five months in the year beginning May 1. His training as Zoölogist and General Physiologist fit him pre-eminently well to serve the Laboratory in his new capacity.

4. Dr. W. J. V. Osterhout who has acted as head of the botanical research staff since 1919 also resigned for the reason that his research program will keep him away from Woods Hole for several years. For the great stimulus of his work and

example the Laboratory is both grateful and proud. Dr. Benjamin Duggar the noted plant physiologist of the Missouri Botanical garden has been elected as worthy successor to Dr. Osterhout.

5. Dr. Robert H. Bowen of Columbia University resigned charge of instruction of the course in Invertebrate Zoölogy after four years of conspicuously successful service in charge, and seven years in all, in order to devote his time more fully to research. Dr. J. A. Dawson of Harvard University an experienced member of the staff of the course for several years was chosen to take charge.

10. *Changes in the By-laws.*—At the meeting of the Board of Trustees, August 12, 1924, a committee composed of the Treasurer, the Secretary and Dr. C. R. Stockard was appointed to consider and revise the By-laws and to report within six months to the Executive Committee. This report was accordingly prepared and presented to the Executive Committee which considered and approved it, and recommended its adoption by the Board of Trustees at their meeting on August 11, 1925. At this time it was considered and finally adopted.

The following Report was presented by the Committee:

Your committee has examined the Certificate of Incorporation, Articles of Association, the original By-laws of the Corporation, and the present By-laws. It finds that the By-laws are in a very simple form and that the absence of elaborate rules has been a benefit to the Corporation, and has made its business easy to administer. It does not recommend, therefore, any general change in the form of the By-laws. There are, however, certain minor changes which should perhaps be made, and to this end the members of the committee are agreed in recommending the following alterations:

1. By-law I. Change last sentence to read as follows:

The President of the Corporation, the Director and Associate Director of the Laboratory shall also be Trustees ex-officio.

2. By-law V. Line 3. Change to read:

They shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose, etc.

3. By-law VII, provides for the appointment annually of two trustees to audit the account of the Treasurer and act as a Finance Committee. The auditing of the Treasurer's accounts has for a number of years been done by certified accountants, and the establishment of the trust funds in the hands of a Trust Company has made it probable that there will be no great amount of investing done by the Treasurer. It would therefore seem that By-law VII should be amended to read as follows:

> The accounts of the Treasurer shall be audited annually by a certified public accountant.

4. By-law VIII. Amend the second sentence to read:

> In case of dissolution the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

These changes are incorporated in the By-laws printed with this report (p. 4). They do not affect in any essential way the original intent of the By-laws but they define more exactly existing practises and provide more broadly for the improbable contingency of dissolution.

11. *The Board of Trustees. Enlargement of the Executive Committee.*—At their meeting held August 11, 1925, the Executive Committee of the Laboratory was enlarged to include three members *ex off.* (the Director, Associate Director and the Treasurer) and four members at large to be elected by the Board from among their own number.

At the meeting of the Board of Trustees held August 11, 1925, Professor Henry E. Crampton of Barnard College, Columbia University was elected to fill a vacancy in the Class of 1927 of the Board. Thus there returns to the service of the Laboratory a noted teacher and investigator whose earlier work on its behalf dates from over thirty years ago.

The following resolution in memory of Lucius L. Nunn former member of the Board of Trustees and benefactor of the Laboratory was adopted by the Board and by the Corporation at their meetings held August 11, 1925:

Resolution on the Death of Mr. L. L. Nunn.

Dr. Lillie offered the following resolution (read at the Corporation meeting) which was ordered spread on the minutes.

Mr. L. L. Nunn, Trustee of the Marine Biological Laboratory from 1897 to 1923 and Life Member of the Corporation died at his home in California, April 2, 1925. A mining engineer by profession, Mr. Nunn attained great success in the development of mines, power lines and banks in the State of Colorado. He was a man of deep human sympathies which found their outlet in his unmarried state in a school for boys, administered on very liberal lines, which he established at Deep Springs in California. In connection with the school scholarships were provided, and assigned on the election of the boys to the Telluride Association, which furnished a complete college and professional education with generous stipend for maintenance. Mr. Nunn also maintained a chapter house at Cornell University named Telluride House after his principal scene of operations in Colorado, for the use of members of the Telluride Association. Many young men owe their prospects in life to these benevolences of Mr. Nunn.

He was a brother-in-law of Professor Whitman, first Director of this Laboratory, and showed constant sympathy with Professor Whitman's ideals and aims. He evinced this interest in a very striking way at the time of reorganization of the administration of the Laboratory in 1897, at which time he was elected to the Board of Trustees. He was present on several critical occasions in the history of the Laboratory and always showed readiness to live up to the responsibilities of his Trusteeship. His largest gift to the Laboratory was a sum of about $5,600.00 in 1902 for the purchase of land important in connection with negotiations with the Carnegie Institution going on at that time.

Although prevented by distance and illness from attending the meetings of the Trustees, Mr. Nunn maintained his interest in its progress. On December 31, 1918 he wrote the Director:

My dear Dr. Lillie: I am just in receipt of your communication under date of December 16th, stating that you will not call a midwinter meeting of the trustees of the laboratory. I think the decision a most wise one and will add one suggestion, viz: that the members of the Board send the laboratory the amount it would cost them to attend the meeting. Acting in accordance with the suggestion, I enclose my check for $200.

Earnestly hoping that I will be able to spend a few weeks with you at Woods Hole the coming summer, I remain

Sincerely,
L. L. NUNN.

Again in August 1923 he telegraphed his regret at his inability to attend the meeting of the Board, and said that his interest in scientific research and devotion to the Laboratory were not lessened, and offered to donate Professor Whitman's collection of pamphlets and documents which were afterwards received.

The Trustees of the Marine Biological Laboratory record their sorrow at the death of their old associate, and direct that this brief record be spread upon the minutes.

12. There are included as parts of this report the following addenda:

1. The Staff, 1925.
2. Investigators and Students, 1925.
3. Tabular view of attendance, 1921–25.
4. Subscribing and Coöperating Institutions, 1925.
5. Evening Lectures, 1925.
6. Members of the Corporation.

I. THE STAFF, 1925.

FRANK R. LILLIE, *Director*, Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.

GILMAN A. DREW, *Assistant Director*, Marine Biological Laboratory.

ZOÖLOGY.

I. INVESTIGATION.

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.

E. G. CONKLIN, Professor of Zoölogy, Princeton University.

CASWELL GRAVE, Professor of Zoölogy, Washington University.

H. S. JENNINGS, Professor of Zoölogy, Johns Hopkins University.

FRANK R. LILLIE, Professor of Embryology, The University of Chicago.

C. E. MCCLUNG, Professor of Zoölogy, University of Pennsylvania.

S. O. MAST, Professor of Zoölogy, Johns Hopkins University.

T. H. MORGAN, Professor of Experimental Zoölogy, Columbia University.

G. H. PARKER, Professor of Zoölogy, Harvard University.

E. B. WILSON, Professor of Zoölogy, Columbia University.

II. INSTRUCTION.

Robert H. Bowen, Assistant Professor of Zoölogy, Columbia University.

Horace B. Baker, Instructor in Zoölogy, University of Pennsylvania.

C. Dale Beers, Graduate Student, Johns Hopkins University.

Rudolf Bennitt, Instructor in Biology, Tufts College.

J. A. Dawson, Instructor in Zoölogy, Harvard University.

Madeleine P. Grant, Assistant Professor, Mount Holyoke College.

Christianna Smith, Assistant Professor of Zoölogy, Mount Holyoke College. (Absent in 1925.)

B. H. Willier, Assistant Professor of Zoölogy, The University of Chicago.

Donnell B. Young, Assistant Professor of Biology, Carleton College

PROTOZOÖLOGY.

I. INVESTIGATION.

(*See Zoölogy.*)

II. INSTRUCTION.

Gary N. Calkins, Professor of Protozoölogy, Columbia University.

Louise H. Gregory, Associate Professor of Zoölogy, Columbia University. (Absent in 1925.)

Florence de L. Lowther, Instructor in Zoölogy, Barnard College.

Mary S. MacDougall, Head of the Biological Department, Agnes Scott College.

EMBRYOLOGY.

I. INVESTIGATION.

(*See Zoölogy.*)

II. INSTRUCTION.

Hubert B. Goodrich, Professor of Biology, Wesleyan University.

Benjamin H. Grave, Professor of Biology, Wabash College.

Charles Packard, Associate in the Institute of Cancer Research, Columbia University.

Harold H. Plough, Associate Professor of Biology, Amherst College.

Charles G. Rogers, Professor of Comparative Physiology, Oberlin College.

PHYSIOLOGY.

I. INVESTIGATION.

Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.

WALTER E. GARREY, Professor of Physiology, Tulane University.

RALPH S. LILLIE, Professor of General Physiology, The University of Chicago.

ALBERT P. MATHEWS, Professor of Biochemistry, The University of Cincinnati.

II. INSTRUCTION.

MERKEL H. JACOBS, Professor of General Physiology, University of Pennsylvania.

WILLIAM R. AMBERSON, Assistant Professor of Physiology, University of Pennsylvania.

FRANK P. KNOWLTON, Professor of Physiology, Syracuse University.

BOTANY.

I. INVESTIGATION.

S. C. BROOKS, Department of Public Health, Washington, D. C.

EDWARD M. EAST, Professor of Experimental Plant Morphology, Harvard University.

ROBERT A. HARPER, Professor of Botany, Columbia University.

E. NEWTON HARVEY, Professor of Physiology, Princeton University.

IVEY F. LEWIS, Professor of Biology, University of Virginia.

WINTHROP J. V. OSTERHOUT, Professor of Botany, Harvard University. (Absent in 1925.)

JACOB R. SCHRAMM, Professor of Botany, College of Agriculture, Cornell University.

II. INSTRUCTION.

IVEY F. LEWIS, Professor of Biology, University of Virginia.

ANSELM M. KEEFE, Fellow in Botany, University of Wisconsin.

WILLIAM RANDOLPH TAYLOR, Assistant Professor of Botany, University of Pennsylvania.

LIBRARY.

PRISCILLA B. MONTGOMERY (Mrs. Thomas H. Montgomery, Jr.), Librarian.

——— ———, Assistant Librarian.

CHEMICAL SUPPLIES.

OLIVER S. STRONG, Associate Professor of Neurology, Columbia University, New York City, Chemist.

APPARATUS ROOM.

Samuel E. Pond, Assistant Professor of Physiology, Medical School, University of Pennsylvania, Philadelphia, Pa. *Custodian of Apparatus.*

SUPPLY DEPARTMENT

George M. Gray, Curator.
Assistant Curator: ——— ———.
John J. Veeder, Captain.
E. M. Lewis, Engineer.
A. W. Leathers, Head of Shipping Department.
A. M. Hilton, Collector.
J. McInnis, Collector.
A. R. Travis, Collector.

F. M. MacNaught, Business Manager.
Herbert A. Hilton, Superintendent of Buildings and Grounds.
Thomas Larkin, Superintendent of Machinery and Plumbing.
Lester F. Boss, Mechanician.
William Hemenway, Carpenter.
Arnold H. Bisco, Storekeeper and head janitor.

2. INVESTIGATORS AND STUDENTS, 1925.

ZOÖLOGY—Independent Investigators.

Addison, William H. F., Professor of Histology and Embryology, University of Pennsylvania.
Baker, Horace B., Instructor, University of Pennsylvania.
Bant, Antoni J., Lemberg University, Poland.
Bennitt, Rudolf, Instructor in Biology, Tufts College.
Bigelow, Robert P., Professor of Zoölogy, Massachusetts Institute of Technology.
Bishop, Mabel, Professor of Zoölogy, Hood College.
Boschma, Hilbrand, Chief Assistant, University of Leyden, Holland.
Bowen, Robert H., Assistant Professor of Zoölogy, Columbia University.
Breitenbecher, Joseph K., Trenton, Ohio.
Bridges, Calvin B., Research Assistant, Carnegie Institution of Washington.
Budington, Robert A., Professor of Zoölogy, Oberlin College.
Calkins, Gary N., Professor of Protozoölogy, Columbia University.
Carothers, E. Eleanor, University of Pennsylvania.
Carver, Gail L., Professor of Biology, Mercer University.
Chambers, Robert, Professor of Microscopic Anatomy, Cornell University Medical College.
Clark, Eliot R., Professor of Anatomy, University of Georgia.
Clark, Eleanor L., Research worker, University of Georgia.
Cleveland, L. R., Fellow, National Research Council.
Conklin, Edwin G., Professor of Biology, Princeton University.
Copeland, Manton, Professor of Biology, Bowdoin College.
Cowdry, Edmund V., Associate Member, Rockefeller Institute.
Danchakoff, Vera, Assistant Professor, College of Physicians and Surgeons.
Dawson, James A., Instructor in Zoölogy, Harvard University.

Dellinger, Samuel C., Professor of Zoölogy, University of Arkansas.
Detlefsen, J. A., Associate Editor, Biological Abstracts.
Dolley, William L., Jr., Assistant Professor of Biology, University of Buffalo.
Forbes, William T. M., Instructor in Entomology, Cornell University.
Fry, Henry J., Assistant Professor, Department of Biology, Washington Square College.
Gabritschevsky, Eugen, Research Fellow, International Education Board.
Glaser, Otto C., Professor of Biology, Amherst College.
Goodrich, Hubert B., Professor of Biology, Wesleyan University.
Grave, Benjamin H., Professor of Zoölogy, Wabash College.
Grave, Caswell, Professor of Zoölogy, Washington University.
Hance, Robert T., Associate Member, Rockefeller Institute.
Hanstrom, Bertil, Assistant Professor, University of Lund, Sweden.
Hess, Walter N., Professor of Zoölogy, DePauw University.
Hoadley, Leigh, Fellow, National Research Council.
Just, Ernest E., Professor of Zoölogy, Howard University.
Kaan, Helen W., 37 Prescott St., Reading, Mass.
Knower, Henry McE., Visiting Professor of Anatomy, University of Georgia.
Lillie, Frank R., Chairman, Department of Zoölogy, University of Chicago.
Lowther, Florence de L., Instructor in Zoölogy, Barnard College.
McClung, Clarence E., Director of Zoölogical Laboratory, University of Pennsylvania.
Martin, Earl A., Assistant Professor, College of the City of New York.
Mast, Samuel O., Professor of Zoölogy, Johns Hopkins University.
Mavor, James W., Professor of Biology, Union College.
Miller, Harry M., Jr., Assistant Professor of Zoölogy, Washington University.
de Mol, W. E., University of Utrecht, Holland.
Morgan, Thomas H., Professor of Experimental Zoölogy, Columbia University.
Morgan, Lilian V., 409 W. 117th St., New York City.
Morrill, Charles V., Associate Professor of Anatomy, Cornell University Medical College.
Nonidez, Jose F., Instructor in Anatomy, Cornell University Medical College.
Packard, Charles, Associate in Zoölogy, Institute of Cancer Research, Columbia University.
Papanicolaou, George N., Assistant Professor in Anatomy, Cornell University Medical College.
Parker, George H., Professor of Zoölogy, Harvard University.
Parmenter, C. L., Assistant Professor, University of Pennsylvania.
Phillips, Everett F., Professor of Agriculture, Cornell University.
Pinney, Mary E., Assistant Professor of Zoölogy, Milwaukee-Downer College.
Plough, Harold H., Professor of Biology, Amherst College.
Rand, Herbert W., Associate Professor of Zoölogy, Harvard University.
Redfield, Helen, National Research Fellow in Zoölogy.
Sanders, Elizabeth P., 2906 St. Paul St., Baltimore, Md.
Sayles, Leonard P., Arnold Fellow, Brown University.
Schrader, Franz, Associate Professor, Bryn Mawr College.
Schrader, Sally H., Instructor, Bryn Mawr College.
Speidel, Carl C., Associate Professor of Anatomy, University of Virginia.
Stark, Mary B., Professor of Histology and Embryology, New York Homœopathic Medical College.

Stern, Curt, Research Fellow, International Education Board.
Stockard, Charles R., Professor of Anatomy, Cornell University Medical College.
Stringer, Caroline E., Head of the Department of Natural Science, Central High School, Omaha, Nebraska.
Strong, Oliver S., Associate Professor of Neurology, Columbia University.
Sturtevant, Alfred H., Member of Staff, Carnegie Institution of Washington.
Thompson, I. Maclaren, Assistant Professor of Anatomy, McGill University.
Uhlenhuth, Edward, University of Maryland, School of Medicine.
Ulrey, Albert B., Professor of Biology, University of Southern California.
Whedon, Arthur D., Professor of Zoölogy, North Dakota Agricultural College.
Wieman, Harry L., Professor of Zoölogy, University of Cincinnati.
Wildman, Edward E., Head of Science Department, West Philadelphia High School.
Willier, Benjamin H., Assistant Professor of Zoölogy, University of Chicago.
Wilson, Edmund B., Da Costa Professor of Zoölogy, Columbia University.
Wilson, J. Walter, Assistant Professor of Biology, Brown University.
Woodruff, Lorande L., Professor of Protozoölogy, Yale University.
Young, Donnell B., Assistant Professor of Biology, Carleton College.
Zeleny, Charles, Professor of Zoölogy, University of Illinois.

ZOÖLOGY—Beginning Investigators.

Anderson, Chas. L., Student, University of Georgia Medical School.
Austin, Mary L., Columbia University.
Baker, Woodford B., Associate Professor of Biology, Emory University.
Baker, Herbert N., Tonganoxie, Kansas.
Beers, Charles D., Johns Hopkins University.
Bergner, A. Dorothy, Investigator, Columbia University.
Blake, Charles H., Assistant, Massachusetts Institute of Technology.
Brown, Alan W., Cornell University Medical College.
Brown, Muriel W., Student, Johns Hopkins University.
Cook, Daniel, University of Cincinnati.
De Forest, David McC., Assistant, Union College.
Fogg, Lloyd C., Graduate Student, Columbia University.
Foster, Kendall W., Instructor in Biology, Tufts Pre-Medical School.
Fraser, Doris A., Assistant, University of Pennsylvania.
Grand, Constantine G., Anatomy Assistant, Cornell University Medical College.
Grant, Madeleine P., Assistant Professor, Mount Holyoke College.
Grave, Thomas B., Johns Hopkins University.
Guerasimoff, Helen, Columbia University.
Harnly, Morris H., Columbia University.
Helwig, Edwin R., Instructor in Zoölogy, University of Pennsylvania.
Hickman, Cleveland P., Assistant Professor of Zoölogy, DePauw University.
Hulpieu, Harold R., Assistant, Johns Hopkins University.
Karns, Hilda E., Cornell University.
Kropp, Benjamin, Graduate Student, Harvard University.
Li, Ju Chi, Columbia University.
Lucas, Alfred M., Assistant Instructor, Washington University.
McClung, Della E., University of Kansas.
McClung, Anna A., 417 Harvard Avenue, Swarthmore, Pa.

MacDougall, Mary S., Head of the Biological Department, Agnes Scott College.
Matthews, Samuel A., Student, Harvard College.
Northrup, Flora E., Washington University.
Parpart, Arthur K., Assistant in Biology, Amherst College.
Philpott, Charles H., Professor of Zoölogy, Harris Teachers College, St. Louis, Missouri.
Plunkett, Charles R., Columbia University.
Sandison, James C., Assistant in Anatomy, Medical Department, University of Georgia.
Schultz, Jack, Assistant in Zoölogy, Columbia University.
Sturtevant, Phoebe R., Assistant, Carnegie Institution.
Thompson, Ruth C. McC., Brick Church, Orange, New Jersey.
Tilden, Evelyn B., Secretary and Laboratory Assistant, Rockefeller Institute.
Varian, Basil B., Laboratory Assistant, University of Pennsylvania.
Vicari, Emilia M., Sweet Briar College.
Wallace, Edith M., Carnegie Institution.
Wierda, J. L., Instructor, Duke University.
Wolff, William A., Greensboro, North Carolina.
Wright, Sydney L., Jr., Chemist, Pennsylvania Hospital.

PHYSIOLOGY—Independent Investigators.

Amberson, William R., Assistant Professor, University of Pennsylvania.
Bazett, Henry C., Professor of Physiology, University of Pennsylvania Medical School.
Berglund, Hilding, Assistant Professor of Medicine, Department of Biochemistry, Harvard University Medical School.
Bodansky, Oscar, 124 East 117th St., New York City.
Bradley, Harold C., Professor of Physiological Chemistry, University of Wisconsin.
Bronfenbrenner, J. J., Associate Member, Rockefeller Institute.
Brunquist, Ernest H., Instructor in Physiology, University of Colorado Medical School.
Cannan, Robert K., Assistant in Biochemistry, University College, London.
Clowes, G. H. A., Director of Research, Eli Lilly & Company.
Cohen, Barnett, Chemist, Hygienic Laboratory, Washington, D. C.
Cohn, Edwin J., Assistant Professor of Physical Chemistry, Harvard University Medical School.
Cracium, Emil C., Assistant, Second Medical Clinic, University of Bucharest, Rumania.
Dembowski, Jan, University of Warsaw.
Dembowski, Mrs. Jan, Warsaw, Poland.
Garrey, Walter E., Professor of Physiology, Vanderbilt University Medical School.
Harvey, Edmund N., Professor of Physiology, Princeton University.
Harvey, Ethel B., 2 College Road, Princeton, New Jersey.
Heilbrunn, L. V., Assistant Professor, University of Michigan.
Hinrichs, Marie A., National Research Council Fellow, University of Chicago.
Inman, Ondess L., Professor of Biology, Antioch College.
d'Irsay, Stephen, Seessel Fellow in Physiology, Yale University.

Irwin, Marian, Research Associate, Rockefeller Institute.
Jacobs, Merkel H., Professor of General Physiology, University of Pennsylvania
Knowlton, Frank P., Professor of Physiology, College of Medicine, Syracuse University.
Lillie, Ralph S., Professor of General Physiology, University of Chicago.
Loeb, Leo, Professor of Pathology, Washington University Medical School.
Lucke, Baldwin, Assistant Professor of Pathology, University of Pennsylvania.
Lyon, Elias P., Professor of Physiology, University of Minnesota.
McCutcheon, Morton, Instructor in Pathology, University of Pennsylvania Medical School.
Mathews, A. P., Professor of Biochemistry, University of Cincinnati Medical School.
Morgulis, Sergius, Professor of Biochemistry, University of Nebraska College of Medicine.
Pond, Samuel E., Assistant Professor of Physiology, University of Pennsylvania School of Medicine.
Raeder, Oscar J., Instructor in Psychiatry, Harvard University.
Redfield, Alfred C., Assistant Professor of Physiology, Harvard University Medical School.
Reznikoff, Paul, Assistant in Anatomy, Cornell University Medical College.
Rogers, Charles G., Professor of Comparative Physiology, Oberlin College.
Sampson, Myra M., Associate Professor, Smith College.
Simpson, George E., Assistant Professor, University of Pennsylvania.
Snyder, Charles D., Professor of Experimental Physiology, Johns Hopkins University.
Warren, Howard C., Stuart Professor of Psychology, Princeton University.

PHYSIOLOGY—Beginning Investigators.

Alexander, Edward G., Fellow in Biology, Princeton University.
Baskervill, Margaret, Instructor, Bellevue Medical College New York City.
Bliss, Chester, I., Graduate Student, Columbia University.
Cattell, Ware, Garrison-on-Hudson, New York.
Coolidge, Thomas, Student, Harvard University Medical School.
Crofts, Elizabeth, Assistant Professor, Mount Holyoke College.
Darby, Hugh H., Instructor, Columbia University.
Genther, Ida T., Student, Mount Holyoke College.
Glusker, David, Investigator, University of Pennsylvania.
Hartline, H. K., Johns Hopkins University Medical School.
Haywood, Charlotte, Graduate Student, University of Pennsylvania.
Hendry, Jessie L., Investigator, Harvard University Medical School.
Hurd, Archer L., Harvard University Medical School.
Kredel, Frederick E., Graduate Assistant in Zoölogy, University of Pittsburgh.
Kupp, John H., Johns Hopkins University Medical School.
Landis, Eugene M., Student, University of Pennsylvania.
Mulder, Arthur G., Teaching Fellow, University of Minnesota.
Page, Irving H., Research worker, Eli Lilly & Company.
Rice, Kenneth S., Graduate Student, Brown University.
Salter, William T., Investigator, Harvard University Medical School.
Stedman, Hazeltene L., Assistant Professor, Mount Holyoke College.

WALDEN, GEORGE B., Research Chemist, Eli Lilly & Company.
WALDEN, MRS. GEORGE B., Chemist, Eli Lilly & Company.

BOTANY—Independent Investigators.

BRIEGER, FRIEDIRCH G., Rockefeller Research Fellow, Bussey Institution.
BROOKS, SUMNER C., Biologist, Hygienic Laboratory, Washington, D. C.
BROOKS, MATILDA M., Associate Biologist, Hygienic Laboratory, Washington, D. C.
CLELAND, RALPH E., Associate Professor of Biology, Goucher College.
DUGGAR, BENJAMIN M., Missouri Botanical Garden, St. Louis, Missouri.
LEWIS, IVEY F., Professor of Biology, University of Virginia.
PRAT, SILVESTER, Assistant, Plant Physiological Laboratory, Charles University.
SCARTH, GEORGE W., Assistant Professor, McGill University.
SCHRAMM, J. R., Editor-in-Chief, Biological Abstracts.
STOKEY, ALMA G., Professor of Botany, Mount Holyoke College.
TAYLOR, WILLIAM RANDOLPH, Assistant Professor, University of Pennsylvania.

BOTANY—Beginning Investigators.

CARGILL, MIRIAM A., Valley Falls, Rhode Island.
KEEFE, ANSELM M., Fellow in Botany, University of Wisconsin.
MILLER, MRS. HARRY, Washington University.
SVENSON, HENRY K., Assistant Professor of Biology, Union College.

STUDENTS.

1925.

Botany.

FLIEDNER, FRIEDA, Teacher, Central High School, Cleveland.
GORDESKY, TOBA, Teacher, Elementary School, Philadelphia, Pa.
GRANT, JEAN F., Teacher, St. Mary's School, Raleigh, N. C.
GRISWOLD, SYLVIA M., Teacher, Maryland State Normal School.
HALL, ADA R., Professor of Biology, Shorter College.
HASTINGS, HELEN, George Washington University.
HORNE, EDITH L., Vassar College.
LIEBERMANN, FANNIE, Science teacher, South Philadelphia High School for Girls.
MCCAFFREY, CHARLES F., JR., Student, Harvard College.
MOORE, IMOGENE, Graduate Student, Yale University.
ROWLEE, SILENCE, Instructor, Elmira College.
TETZLOFF, LINA E., Instructor, Hunter College.
TURNER, RICHARD G., Knoxville, Tennessee.

Embryology.

ARMSTRONG, PHILIP B., Assistant in Anatomy, Cornell University Medical College.
BUNTEN, ISABEL, Student, George Washington University.
CAMPBELL, MALCOLM D., Junior Master, Dorchester High School.
CLARKE, HENRY G., Student, Amherst College.
COBB, WILLIAM M., Amherst College.
DETTMER, CLARA R., Elmira College.
DOOLEY, EUNICE J., Student, Illinois Wesleyan University.

FABING, HOWARD D., Student, University of Cincinnati.
FRIEDMAN, HILDA, Goucher College.
GELBACK, ELIZABETH L., Student, Goucher College.
GREENBERG, FRANCES, New York University.
HALSEY, HARVEY R., Graduate student and laboratory assistant, Columbia University.
HINTZE, ANNA LAURA, Assistant, University of Wisconsin.
HOLTON, RUTH G., Laboratory assistant, Wilson College.
JOHNSON, HELEN R., Assistant in Biology, Brown University.
KOSTIK, JOHN, Student, University of Wisconsin.
LABARRE, ELIZABETH R., Student, Goucher College.
LEICH, CHARLES F., Wabash College.
LONDON, ISABEL M., Cornell University Medical College.
MARKS, EDITH E., Student, Vassar College.
MORRISON, HAROLD, Associate Entomologist, U. S. Bureau of Entomology.
PALLETTE, EDWARD C., Leland Stanford University.
PATTERSON, EDNA, Assistant Professor of Biology, Wesleyan College.
PEARSON, NATHAN E., Assistant in Zoölogy, Indiana University.
PYLE, MARY M., Graduate Assistant, University of Pittsburgh.
THOMAS, MORRIS C., Wabash College.
WARTERS, MARY, Ohio State University.
WHEELER, PHILIP H., Wesleyan University.
WILLIAMS, ELIZABETH T., Smith College.

Physiology.

BRANHAM, SARA E., Instructor in Bacteriology, The University of Chicago.
CAMPOS, F. A. DEM., Assistant in Physiology, St. Pauls Medical School, Brazil.
CHEN, TSE-YIN, Instructor in Biology, Peking University.
CURRY, LALIAH F., Zoölogy Assistant, Washington University.
DEGRAFF, ARTHUR C., Crete Research Fellow in Physiology, Western Reserve University.
DILLER, WILLIAM F., JR., Instructor in Zoölogy, University of Pennsylvania.
EMERSON, ETHEL, Student, College of Physicians and Surgeons.
GRAHAM, ELEANOR P., Baltimore, Maryland.
HERRICK, RUTH, University of Chicago.
MILLER, RALPH E., Medical Student, Dartmouth College.
NEAL, MARY E., Teacher of Science, Boston Schools.
RAFFEL, DANIEL, Graduate Student, Johns Hopkins University.
SANDS, JANE, Professor of Physiology, Woman's Medical College of Pennsylvania.
SCOTT, WALTER J., University of Pennsylvania.
STEWART, DOROTHY R., Hanover, New Hampshire.
SUMWALT, MARGARET, Washington University.
WEIMER, BERNAL R., Professor of Biology, Bethany College.
WILLIAMS, HARLEY A., Oberlin College.
YOUNG, WILLIAM C., Assistant in Department of Biology, Amherst College.

ATTENDING MORNING LECTURES ONLY.

BART, MAX S., 1685 University Ave., New York City.

Protozoölogy.

Banks, Reba, Kirbyville, Texas.
Burt, Frederick A., Associate Professor, A. and M. College of Texas.
Coleman, Peter F., 3314 Cortelyou Road, Brooklyn, New York.
Cooper, Zola K., Graduate Student, Washington University.
Gorham, Grace V., Assistant in Zoölogy, Columbia University.
Kamenoff, Ralph J., Columbia University.
Kempton, Rudolf T., Assistant, Washington Square College, New York University.
Lichtman, Frieda, Columbia University.
Maxwell, Reginald D., Senior Assistant in Biology, Amherst College.
Pan, Quentin, Shanghai, China.
Pierce, Madelene E., Radcliffe College.
Rice, Lucinda H., Assistant Instructor, Wellesley College.
Robert, Nan L., Professor of Biology, Flora Macdonald College.
Silberblatt, Beryl, Student, Columbia University.
Spalding, Janet E., Laboratory Assistant, College of the City of New York.
Van Duyn, Evelyn, Science Teacher, New York City.
Zeman, Mira, Assistant in Biology, New York University.

Attending Morning Lectures Only.

Keith, Wilma, 1229 Carr Avenue, Memphis, Tennessee.

Zoölogy.

Andrews, Cecil L., Butler College.
Bauer, Ethel C., H. Sophie Newcomb Memorial College.
Booth, Lois, DePauw University.
Bosworth, Edward B., Wesleyan University.
Buehler, Katherine, Teacher of Biology, Albany High School.
Carpenter, Esther, Ohio Wesleyan University.
Castle, William A., University of Chicago.
Chamberlain, Harry, University of Rochester.
Conklin, Cecile L., Teacher of Biology, Sweet Briar College.
Corrington, Julian D., Professor of Zoölogy, University of South Carolina.
Crosby, Marion R., Student, University of Wisconsin.
Curtis, Mary E., Student, Wilson College.
Dahlberg, Ruth M., Knox College.
Dempster, Wilfrid T., Student, University of Michigan.
Fales, Doris E., Assistant in Biology, Western Reserve University.
Fisher, Carol A., Graduate Student, Yale University.
Fleming, Joan, Student, Wellesley College.
Franz, Gerhard E., Instructor of Zoölogy and Chemistry, Watertown College.
Friend, Roger B., Graduate Student, Yale University.
Getchell, Donnie C., Laboratory Assistant, Colby College.
Gordon, Kenneth L., Graduate Assistant, University of Missouri.
Gregersen, Magnus I., Teaching Fellow, Harvard Medical School.
Hardy, Jerry D., Professor of Biology, High Point College.
Hendee, Esther C., Oberlin College.

Hitchcock, Harold B., Student, Williams College.
Hitchcock, Pauline D., Assistant Instructor in Biology, Simmons College.
Humeston, Elizabeth, Elmira College.
Hunt, Arthur, Carleton College.
Hutchinson, George A., Student, University of Rochester.
Isely, Marion F., Graduate Assistant, University of Missouri.
Johnson, Willis H., Wabash College.
Kinney, Elizabeth T., Assistant in Zoölogy and Graduate Student, Washington University.
Kirk, Margaret B., Radcliffe College.
Knappen, Phoebe M., George Washington University.
Ladd, Carolyn T., University of Vermont.
Leach, Harriet P., Smith College.
Levin, Paul M., Johns Hopkins University.
Long, Margaret E., Student, University of Pennsylvania.
Miller, Helen M., Student, Goucher College.
Mitani, Fumiko, Student, Mt. Holyoke College.
Mustin, Margaret E., Assistant in Biology, Shorter College.
Quinn, Irene C., Carleton College.
Ryerson, Rockwell T., Northwestern University.
Sarles, Merritt P., Graduate Assistant in Biology, Wesleyan University.
Schuett, Jesse F., St. Louis, Mo.
Shevket, Fazile, Laboratory Assistant, Constantinople College.
Taylor, Phillis, Hunter College.
TeWinkel, Helen McG., Student, Oberlin College.
Tyler, A. Ranger, Student, Rutgers College.
Van Meter, Irene, Student, Illinois Wesleyan University.
Weatherford, Harold L., Austin Teaching Fellow in Histology, Harvard University.
Weatherby, Jesse H., Graduate Student, Johns Hopkins University.
Weichert, Charles K., Assistant in Zoölogy, University of Wisconsin.
Welsh, John H., Jr., Harvard University.

3. TABULAR VIEW OF ATTENDANCE.

	1921	1922	1923	1924	1925
Investigators—Total	172	182	176	194	207
Independent:					
Zoölogy	79	87	90	77	84
Physiology	26	28	23	33	40
Botany	13	15	13	14	11
Under Instruction:					
Zoölogy	34	34	41	47	45
Physiology	11	11	5	16	23
Botany	9	7	4	7	4
Students—Total	120	126	146	134	132

Zoölogy	53	59	59	50	54
Protozoölogy	11	12	16	17	17
Embryology	28	28	31	29	29
Physiology	18	19	22	18	19
Botany	10	8	18	20	13
TOTAL ATTENDANCE	292	308	322	328	340
INSTITUTIONS REPRESENTED—Total	95	104	107	110	112
By investigators	67	71	62	69	74
By students	53	68	73	68	65
SCHOOLS AND ACADEMIES REPRESENTED					
By investigators	—	—	—	—	1
By students	—	—	4	—	4

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS, 1925.

Amherst College
Antioch College
Biological Abstracts
Bowdoin College
Brown University
Bryn Mawr College
Buffalo Society of Natural Sciences
Butler College
Carnegie Institution of Washington
Chinese Educational Mission
College of Physicians & Surgeons
Columbia University
Cornell University
Cornell University Medical College
Dartmouth College
DePauw University
Elmira College
General Education Board
Goucher College
Harvard University
Harvard University Medical School
High Point College
Hunter College
Indiana University
International Education Board
Johns Hopkins University
Johns Hopkins University Medical School
Knox College
Eli Lilly & Co.
Massachusetts Institute of Technology
McGill University
Mount Holyoke College
New York University
Oberlin College
Ohio Wesleyan University
Princeton University
Radcliffe College
Rockefeller Foundation
Rockefeller Institute for Medical Research

Rutgers College
Simmons College
Smith College
Sophie Newcomb College
Trinity College
Tufts College
Union College
University of Chicago
University of Cincinnati
University of Illinois
University of Michigan
University of Minnesota
University of Missouri
University of Nebraska
University of Pennsylvania
University of Pennsylvania Medical School
University of Rochester
University of Vermont
University of Virginia
University of Wisconsin
Vassar College
Wabash College
Washington University
Washington University Medical School
Watertown College
Wellesley College
Wesleyan University
Western Reserve University
Wilson College
Wistar Institute of Anatomy and Biology
Yale University

SCHOLARSHIP TABLES.

The Lucretia Crocker Scholarships for Teachers in Boston, since 1888.

Scholarship of $100.00, supported by a Friend of the Laboratory, since 1898.

The Edwin S. Linton Memorial Scholarship of Washington and Jefferson College.

5. EVENING LECTURES, 1925.

Friday, July 3,
Professor W. J. V. Osterhout....“Absorption and Accumulation.”

Tuesday, July 7,
Professor G. H. Parker..........“The Carbon Dioxide Excreted by Nerves.”

Friday, July 10,
Professor E. B. Wilson..........“Protoplasmic Systems and Genetic Continuity”—Illustrated.

Tuesday, July 14,
Professor G. N. Calkins.........“Organization and Variation in Protozoa.”

Friday, July 17,
Dr. F. M. Chapman...............“The Origin and Distribution of the Birds of Ecuador”—Illustrated.

Tuesday, July 21,
Professor C. R. Stockard........“Analysis and General Bearings of the Ovulation Problem.”

Friday, July 24,
Dr. William Crocker.............“Boyce Thompson Institute for Plant Research and Its Work”—Illustrated.

Tuesday, July 28,
Professor H. E. Crampton ....“Among the Islands of the South Seas”—Illustrated.

Friday, July 31,
Dr. Sewall Wright...............“Genetic Studies with Three- and Four-toed Races of Guinea Pigs.”

Tuesday, August 4,
Professor E. Newton Harvey....“Animal Luminescence.”

Friday, August 7,
Professor Ross G. Harrison......“Heteroplastic Grafting.”

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6. MEMBERS OF THE CORPORATION.

1. Life Members.

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Clarke, Prof. S. F., Williamstown, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Crane, Mr. C. R., New York City.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Folsom, Miss Amy, 88 Marlboro St., Boston, Mass.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.

Gardiner, Mrs. E. G., Woods Hole, Mass.
Gardiner, Miss Eugenia, 15 W. Cedar St., Boston, Mass.
Harrison, Ex-Provost C. C., University of Pennsylvania, Philadelphia, Pa.
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Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
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Mason, Miss Ida M., 1 Walnut St., Boston, Mass.
Means, Dr. James Howard, 15 Chestnut St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.
Morgan, Prof. T. H., Columbia University, New York City, N. Y.
Morgan, Mrs. T. H., New York City, N. Y.
Noyes, Miss Eva J.
Osborn, Prof. Henry F., American Museum of Natural History, New York City, N. Y.
Phillips, Mrs. John C., Windy Knob, Wenham, Mass.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pa.
Pulsifer, Mr. W. H., Newton Center, Mass.
Sears, Dr. Henry F., 86 Beacon St., Boston, Mass.
Shedd, Mr. E. A.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, N. Y.
Trelease, Prof. William, University of Illinois, Urbana, Ill.
Ware, Miss Mary L., 41 Brimmer St., Boston, Mass.
Williams, Mrs. Anna P., 505 Beacon St., Boston, Mass.
Wilson, Dr. E. B., Columbia University, New York City, N. Y.
Wilson, Prof. W. P., Commercial Museum, Philadelphia, Pa.

2. REGULAR MEMBERS, AUGUST, 1925.

ADAMS, MISS A. E., Mount Holyoke College, South Hadley, Mass.

ADDISON, DR. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pa.

ADOLPH, DR. EDWARD F., University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

AGERSBORG, DR. H. P. K., James Millikin University, Decatur, Illinois.

ALLEE, DR. W. C., University of Chicago, Chicago, Illinois.

ALLEN, PROF. CHAS. E., University of Wisconsin, Madison, Wisconsin.

ALLEN, PROF. EZRA, Ursinus College, Collegeville, Pa.

ALLYN, DR. HARRIET M., Vassar College, Poughkeepsie, N. Y.

AMBERSON, DR. WILLIAM B., University of Pennsylvania, Philadelphia, Pa.

ANDERSON, DR. E. G., University of Michigan, Ann Arbor, Michigan.

ATTERBURY, MRS. RUTH R., Great Neck, Long Island, New York.

BAITSELL, DR. GEORGE A., Yale University, New Haven, Conn.

BAKER, DR. E. H., 5312 Hyde Park Boulevard, Hyde Park Station, Chicago, Ill.

BALDWIN, DR. F. M., Iowa State College, Ames, Iowa.

BASCOM, DR. K. F., Medical School of Virginia, Richmond, Virginia.

BECKWITH, DR. CORA J., Vassar College, Poughkeepsie, N. Y.

BEHRE, DR. ELINOR H., Louisiana State University, Baton Rouge, La.

BENNITT, DR. RUDOLF, Tufts College, Tufts College, Mass.

BIGELOW, PROF. M. A., Teachers College, Columbia University, New York City.

BIGELOW, PROF. R. P., Massachusetts Institute of Technology, Cambridge, Mass.

BINFORD, PROF. RAYMOND, Guilford College, Guilford College, N. C.

BORING, DR. ALICE M., Yenching College, Peking, China.

BOWEN, DR. ROBERT H., Columbia University, New York City.

Box, Miss Cora M., University of Cincinnati, Cincinnati, Ohio.
Bradley, Prof. Harold C., University of Wisconsin, Madison, Wis.
Brailey, Miss Miriam E., 232 Maple St., Lynn, Mass.
Bridges, Dr. Calvin B., Columbia University, New York City.
Brooks, Dr. S. C., U. S. Public Health Service, Washington, D. C.
Buckingham, Miss Edith N., 342 Marlboro St., Boston, Mass.
Budington, Prof. R. A., Oberlin College, Oberlin, Ohio.
Bumpus, Prof. H. C., Brown University, Providence, R. I.
Byrnes, Dr. Esther F., 193 Jefferson Ave., Brooklyn, N. Y.
Calkins, Prof. Gary N., Columbia University, New York City.
Calvert, Prof. Philip P., University of Pennsylvania, Philadelphia, Pa.
Carlson, Prof. A. J., University of Chicago, Chicago, Illinois.
Carothers, Dr. Eleanor E., University of Pennsylvania, Philadelphia, Pa.
Carroll, Prof. Mitchel, Franklin and Marshall College, Lancaster, Pa.
Carver, Prof. Gail L., 613 Orange St., Macon, Georgia.
Casteel, Dr. D. B., University of Texas, Austin, Texas.
Cattell, Prof. J. McKeen, Garrison-on-Hudson, New York.
Cattell, Dr. McKeen, Cornell University Medical College, New York City.
Cattell, Mr. Ware, Garrison-on-Hudson, New York.
Chambers, Dr. Robert, Cornell University Medical College, New York City.
Charlton, Dr. Harry H., University of Missouri, Columbia, Missouri.
Chidester, Prof. F. E., West Virginia University, Morgantown, W. Va.
Child, Prof. C. M., University of Chicago, Chicago, Illinois.
Clapp, Prof. Cornelia M., Montague, Mass.
Clark, Prof. E. R., University of Georgia, Augusta, Georgia.
Cleland, Prof. Ralph E., Goucher College, Baltimore, Maryland.
Clowes, Dr. G. H. A., Eli Lilly & Co., Indianapolis, Indiana.
Coe, Prof. W. R., Yale University, New Haven, Conn.

Cohn, Dr. Edwin J., 43 Kirkland St., Cambridge, Mass.

Coker, Dr. R. E., University of North Carolina, Chapel Hill, North Carolina.

Cole, Dr. Leon J., College of Agriculture, Madison, Wisconsin.

Collett, Dr. Mary E., Western Reserve University, Cleveland, Ohio.

Colley, Mrs. Mary W., 1712 Madison St., Madison, Wisconsin.

Colton, Prof. H. S., Ardmore, Pennsylvania.

Coolidge, Mr. C. A., Ames Building, Boston, Mass.

Copeland, Prof. Manton, Bowdoin College, Brunswick, Maine.

Cowdry, Dr. E. V., Rockefeller Institute, New York City.

Crampton, Prof. H. E., Barnard College, Columbia University, New York City.

Crane, Mrs. C. R., Woods Hole, Mass.

Curtis, Prof. W. C., University of Missouri, Columbia, Mo.

Curtis, Dr. Maynie R., Crocker Laboratory, Columbia University, New York City.

Danchakoff, Dr. Vera, College of Physicians and Surgeons, New York City.

Davis, Dr. Donald W., College of William and Mary, Williamsburg, Va.

Davis, Dr. Alice R., 19 Ash St., Cambridge, Mass.

Dawson, Dr. J. A., Harvard University, Cambridge, Mass.

Dederer, Dr. Pauline H., Connecticut College, New London, Connecticut.

Detlefsen, Dr. J. A., Swarthmore, Pennsylvania.

Dexter, Dr. J. S., University of Porto Rico, Rio Piedras, Porto Rico.

Dodds, Prof. G. S., Medical School, University of West Virginia, Morgantown, W. Va.

Dolley, Prof. William L., University of Buffalo, Buffalo, N. Y.

Donaldson, Prof. H. H., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

Donaldson, Dr. John C., University of Pittsburgh, School of Medicine, Pittsburgh, Pa.

Drew, Prof. Gilman A., Eagle Lake, Florida.

Duggar, Dr. Benjamin M., Missouri Botanical Garden, St. Louis, Missouri.

Dungay, Dr. Neil S., Carleton College, Northfield, Minn.

Dunn, Dr. Elizabeth H., Woods Hole, Mass.

Edwards, Dr. D. J., Cornell University Medical College, New York City.

Eigenmann, Prof. C. H., University of Indiana, Bloomington, Ind.

Ellis, Dr. F. W., Monson, Mass.

Farnum, Dr. Louise W., Hunan-Yale Hospital, Changsha, Hunan, China.

Field, Miss Hazel E., University of California, Berkeley, California.

Finley, Mr. Charles W., Lincoln School, 425 West 123d St., New York City.

Forbes, Dr. Alexander, Harvard University Medical School, Boston, Mass.

Fry, Mr. Henry J., Washington Square College, New York City.

Gage, Prof. S. H., Cornell University, Ithaca, N. Y.

Garrey, Prof. W. E., Vanderbilt University Medical School, Nashville, Tennessee.

Gates, Prof. R. Ruggles, King's College, London, England.

Geiser, Dr. S. W., Southern Methodist University, Dallas, Texas.

Glaser, Prof. O. C., Amherst College, Amherst, Mass.

Glaser, Prof. R. W., Rockefeller Institute for Medical Research, Princeton, N. J.

Goldforb, Prof. A. J., College of the City of New York, New York City.

Goodrich, Prof. H. B., Wesleyan University, Middletown, Connecticut.

Gowanloch, Dr. J. N., Dalhousie University, Halifax, Nova Scotia.

Grave, Prof. Caswell, Washington University, St. Louis, Mo.

Grave, Prof. B. H., Wabash College, Crawfordsville, Ind.

Greenman, Dr. M. J., Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania.

Gregory, Dr. Louise H., Barnard College, Columbia University, New York City.

Guthrie, Dr. Mary J., University of Missouri, Columbia, Mo.

Guyer, Prof. M. F., University of Wisconsin, Madison, Wis.

Hance, Dr. Robert T., Rockefeller Institute, New York City.

Hargitt, Prof. C. W., Syracuse University, Syracuse, New York.

Hargitt, Prof. George T., Syracuse University, Syracuse, New York.

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BIOLOGICAL BULLETIN

OBSERVATIONS ON MOTILITY IN CERTAIN CYANOPHYCEÆ.[1]

EDWARD S. CASTLE.

The power of spontaneous movement in the blue-green algæ has been stated by many authors to be confined to the family Oscillatoriaceæ, occurring characteristically in the vegetative filaments of *Oscillatoria*, *Phormidium*, *Spirulina* and *Arthrospira*, and occasionally in the hormogonia of related genera and even of such Heterocysteæ as *Nostoc*, *Scytonema*, and *Rivularia*. In addition, DeBary (1863) and Phillips (1904) have described motion in vegetative filaments of the genus *Cylindrospermum*, a member of the Nostocaceæ.

Recently, however, two species of *Anabæna* occurring on the estate of Mr. E. F. Atkins at Soledad, Cuba, and studied by the writer in the Harvard Laboratory there, were found, although belonging to the Nostocaceæ, to show consistent motility like that characteristic of the Oscillatoriaceæ. These two species were both commonly collected, forming thin scums on damp earth in roadside ditches. In culture bottles they spread rapidly up the sides of the glass in a manner resembling an active *Oscillatoria*, finally covering the whole inside surface with filaments packed side by side in a single layer. Although exhibiting an active motility like that regarded as characteristic of members of the Oscillatoriaceæ, the filaments of both these species were torulose and consistently contained heterocysts that conclusively excluded them from that family. The larger of the two forms (Fig. 1*b*) regularly produced spores and in all essential characteristics justified being identified as a species of *Anabæna;* the other, smaller form (Fig. 1*a*), although it never developed spores during the two months it was under observation, nevertheless was judged an *Anabæna* on its general morphology. Exact determination of the species was not attempted by the writer but will be referred later to specialists in the classification of the group.

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[1] Studies from the Biological Laboratory in Cuba (Atkins Foundation) of the Harvard Institute for Tropical Biology and Medicine, No. 1.

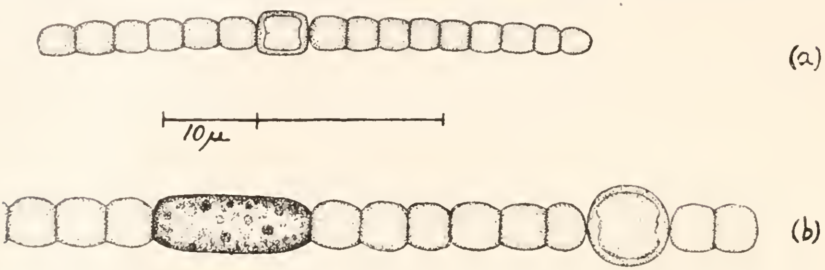

Fig. 1. Portions of the trichomes of the motile species of *Anabæna* studied at Soledad. (*a*) The smaller species showing its characteristic structure. (*b*) The larger species showing the cells, heterocyst, and spore in their characteristic arrangement.

Both these species of *Anabæna* were consistently and actively motile, the motility resembling in general that customary in the Oscillatoriaceæ. Flexion of the whole filament as well as translatory movement was seen in both species, but rotation was not observed. Filaments with heterocysts moved as actively as those without, contrary to what Borzi (1886) observed in the case of species of *Nostoc*. Speed of translation was, within limits, independent of the length of the filament, confirming the observations of Crozier and Federighi (1924) on *Oscillatoria*. At 28.9° C. (84° F.), the rates of translation of these species of *Anabæna*, compared with the rates of some rapidly-motile species of *Oscillatoria*, were:

Anabæna sp.	av. 2.5 μ per sec.
Oscillatoria formosa	" 4.2 μ " "
Oscillatoria princeps	" 5.0 μ " "

The rate of movement in *Anabæna* is thus seen to be half that of *Oscillatoria princeps* and somewhat more than half that of *Oscillatoria formosa* under the same conditions. Although motility has not been reported in *Anabæna* hitherto, the motility which has been recorded frequently in the hormogonia or such related Heterocysteæ as *Nostoc*, *Scytonema*, or *Rivularia* has invariably been described as of a very slow nature. The rates given by West (1916, p. 23), as found by Brand for the hormogonia of species of *Phormidium*, vary from 0.004 to 0.058 μ per second, and are thus many times slower than those seen by the writer in the vegetative filaments of *Anabæna;* but it should be noted that the temperature at which these slower rates occurred was not given.

In view of the fact that during the movement of these species of *Anabæna* no rotation was ever observed by the writer, it seemed advisable for comparison to examine species of *Oscillatoria* in this respect, especially since Crozier and Federighi (*loc. cit.*) have expressed some doubt whether rotation of the filaments of *Oscillatoria* does indeed always take place during translation. Careful examination of a considerable number of favorable species of *Oscillatoria* at Soledad has convinced the writer most conclusively that many species do exhibit rotation of the filament around the long axis. In species in which the ends of the filaments were characteristically hooked, this hook, which maintained its peculiar shape, could be seen to rotate through the water in a manner precluding a mere circular bending of the tip. Moreover, in larger species, rotation could be observed very convincingly by following the change in relative position of granules lying at different depths within the cell content.

Also, in view of the fact that West (1916 and 1904), when discussing the motility of *Arthrospira*, states that rotation has not been observed in this genus, it may be of interest to record that in a species studied at Soledad, rotation was found to accompany progression, as in *Spirulina*, while flexion also was seen to occur at times.

SUMMARY.

Two species of *Anabæna*, of the family Nostocaceæ, studied in Cuba, were found to exhibit motion similar to that of the Oscillatoriaceæ. The rate of movement was independent of the presence of heterocysts or of the length of the filament, and was about one half that of actively motile Oscillatoriæ under the same conditions, but much more rapid than that previously recorded for the hormogonia of *Phormidium*. No rotation was observed in these species of *Anabæna* but several species of

Oscillatoria and one species of *Arthrospira* were seen to rotate customarily during free-swimming translation when observed under similar conditions.

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NUMBER AND BEHAVIOR OF THE CHROMOSOMES IN *CAVIA COBAYA*[1] (THE COMMON GUINEA PIG).

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Introduction.

Since the guinea pig has been used extensively in genetic experiments and since investigation has revealed in many instances a close correlation between the genetic behavior and the number and behavior of the chromosomes of many organisms, the number and behavior of the chromosomes of the guinea pig is of interest alike to the cytologist and the geneticist.

According to Harvey (1920) there is a wide difference of opinion concerning the number of chromosomes observed in *Cavia*. Von Bardeleben (1892) reports sixteen spermatogonial chromosomes and eight chromosomes in the spermatids. Flemming (1898) says that there are probably twenty-four somatic chromosomes. Moore and Walker (1906) give the number as thirty-two in the spermatogonial cells and sixteen in the primary and secondary spermatocyte cells. Stevens (1911) says that there are about fifty-six spermatogonial chromosomes and twenty-eight chromosomes in both the primary and secondary spermatocytes. She further states that the sex chromosomes are of the XY-type which are unequal in size and separate in the first spermatocyte division. Athias (1912) reports twenty-four to twenty-eight primary spermatocyte chromosomes in *Cavia porcellus* and perhaps twenty-four secondary spermatocyte chromosomes. Lams (1913) says that there are sixteen somatic chromosomes, and eight primary and secondary spermatocyte chromosomes and eight chromosomes in the spermatids. In most cases the material has been reported as very unfavorable or the count doubtful. Our counts do not agree with any of those reported previously. We may add, however, that in many instances our material is almost diagrammatically clear. We have found thirty-eight spermatogonial chromosomes and nineteen chromosomes in the primary and secondary spermatocyte cells. Like Stevens, we have found an XY-chromosome which separates in the first maturation division.

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[1] Dr. H. L. Ibsen furnished the guinea pigs for this study. P. W. Gregory assisted in the fixation and dehydration. The drawings were made by S. Fred Prince.

[2] Contribution from the Zoölogical Laboratory, Kansas State Agricultural College No. 851.

Material and Methods.

The present paper is limited to the number and behavior of the spermatogonial and spermatocyte chromosomes. We have found some interesting things in connection with the transformation of the spermatid which we hope to give in another paper.

Two male guinea pigs were used for this study. One of these animals was superactive sexually and the other was a normally active male. The testes were removed and cut into small sections. These were placed in cold Flemming's solution to which urea had been added. This was done according to the technique of Hance (1917) with the exception that the temperature was 2° C. instead of 4° C. The fixing fluid had been out of doors several hours previous to using it. The material was fixed for about twenty-four hours. The temperature was almost constant during this time. The pieces of fixed testes were transferred to water at room temperature for twenty-four hours. The water was changed frequently. Dehydration was gradual, although the drop method was not employed. Xylol was used for clearing and paraffin was the embedding medium. The sections were cut from three to ten micra. Those sections from three to seven micra proved best for study. It was difficult to isolate individual cells in thicker sections. Sections three micra did not seem to be particularly advantageous over those five or six micra. The most favorable stain was Heidenhain's iron-alum hematoxylin.

Description of Material.

It was found that the actively dividing cells are not distributed throughout the whole lining of the seminiferous tubules but are in very restricted areas. These areas are larger and more numerous in the superactive male. They are elliptical in shape. The greatest diameter is always lengthwise of the tubule. This dimension never exceeds two thirds of the circumference of the tubule and may be as small as only a few cells.

1. *Spermatogonial Divisions.*

Many and repeated counts have given us thirty-eight spermatogonial chromosomes. Eight of these are distinctly U-shaped which may be arranged in four pairs. Twenty-eight chromosomes are slightly bent rods of various sizes which, according to size and shape, may be arranged in pairs. The remaining two chromosomes are unequal in size and irregular in shape. We have designated these as the XY-pair. Fig. 2 is a polar view of a metaphase plate of a spermatogonial division. The chromosomes which form the members of a given pair have been designated by the same number. Reference to this figure shows that the chromosomes are not arranged merely around the periphery of the spindle as is so often the case in insects (Harman, 1915 and 1920), but fill in the entire diameter of the spindle. No one kind of chromosome occupies one area of the spindle exclusively. The large, slightly bent rods are deep in the spindle as well as around the periphery. The same may be said of the U-shaped chromosomes. However, the relative arrangement of these various chromosomes is approximately the same.

2. *The Growth Period.*

Evidently a longer period of time is spent in the stages between the last spermatogonial division and the first spermatocyte division than in any other stage, for there are always many times more cells in these stages than the other stages. This is true, not only for the animals used in this study, but also for other guinea pigs which we have examined.

In the early prophase of the primary spermatocyte cells the chromatin is coarsely granular and is in an indistinct reticulum. There is no uniformity in either the size or the shape of these granules in a particular cell, but there are always a few which are distinctly larger than the majority and decidedly more compact. As the nucleus increases in size, the chromatin granules become smaller and the reticulum less distinct until the nucleus is almost clear, except for here and there masses of chromatin of loose construction. After this the nucleus does not get much larger but the chromatin material becomes more concentrated near one side of the nucleus until it becomes a tangled mass in which it is difficult to distinguish any particular parts or any definite arrangement. Gradually this mass loosens up and more nearly fills the entire nucleus. In addition to the reticulum there is a number of more compact particles of chromatin material. These seem to be the beginnings of the bivalent chromosomes. Long before all the chromatin material has been formed into definite chromosomes each of these masses appears double. Fig. 3 shows nineteen of these double chromosomes with the chromatin in so loose a condition that each body has a woolly appearance. Fig. 5 is a later stage in which the chromatin is more compact and the double condition is no longer apparent. Figs. 4 and 6 are fully formed primary spermatocyte chromosomes. By reference to these figures it will be seen that no two chromosomes are of exactly the same shape or size. As was found in the spermatogonial divisions the spermatocyte chromosomes are distributed through the entire diameter of the spindle.

3. *Spermatocyte Divisions.*

Figure 7 is a lateral view of a metaphase plate of a primary spermatocyte division. It will be noted that one chromosome is almost divided and that the division is an unequal one. We have thought that this is the XY-chromosome. We have designated the larger component as the X and the smaller one as the Y. Fig. 8 shows the autosomes undivided while the XY has completed its division and each component is half way to the poles. Fig. 10 is an anaphase of a primary spermatocyte division. This figure shows a lack of synchronism in the division of the autosomes.

Figure 9 is an anaphase of a secondary spermatocyte division. It will be seen that some of the chromosomes divide much in advance of the others. Figs. 11 and 12 are polar views of metaphase plates of the secondary spermatocyte divisions. In each of these figures are seen nineteen chromosomes. Fig. 12 is not a direct polar view but is a view of the metaphase plate at a tangent.

Discussion.

The large number of chromosomes, the comparative small size and the tendency of the chromatin material to mass together and form into clumps in which the individual parts are not distinguishable have made the study of mammalian chromosomes a difficult task. This may, in part, account for the differences in the interpretation of the chromosome complex in observations on the same species as was cited in the beginning of this paper. Other difficulties are the scarcity of cells in the various stages of active division, the lack of synchronism in the division of the chromosomes and the distribution of the chromosomes throughout the entire diameter of the spindle.

We are of the opinion that a selection of animals which show sexual activity at the time of securing the material greatly increases the size and number of areas of cells in active stages of division. We found this true not only for the animals used in this study but also for a number of other guinea pigs which we have observed. This also agrees with Painter's (Painter, 1924) observations on the monkey and other mammals.

Since the chromosomes are closely packed together the selection of a fixing agent which does not swell the chromatin material is very desirous. We have found that the modification of Flemming's solution with urea used at a temperature slightly above freezing gives the most satisfactory results. We have used Allen's modification of Bouin (Allen, 1919) at about body temperature with less satisfactory results. This is the technique which Painter has used so successfully.

Most of the cells in the actively dividing stages of our material are diagrammatically clear. Owing to the fact that some of the chromosomes are precocious and others are lagging, many of the polar views of metaphase plates do not contain all the chromosomes.

It is of some interest to note that although there is a great difference in the number of chromosomes reported for the guinea pig, no one has reported an unpaired chromosome and only Stevens (1911) has previously reported one which divides unequally. This is the condition which Painter (1923 and 1924) has found for the opossum, two species of monkeys, the horse and both the negro and the white man. However, it differs from the findings of Wodsedalek (1914) and Masui (1919) for the horse. Since the female has not been studied the basis for calling this pair of unequally dividing chromosomes the XY-pair is the behavior. We have chosen to call the large component the X-element and the smaller component the Y-element because this is the condition in those animals in which the homologues have been studied in the female. It is perfectly possible that an examination of the female might reveal the opposite condition. This is of further interest because it is cytological evidence that the male guinea pig is heterozygous for sex which has been taken for granted among geneticists but which has not been shown experimentally. Known sex-linked characters in the mammals are few. They have been found in man and the cat.

We have found no indication of telosynapsis as was described by Moore and Walker (1906). They state that "In the guinea pig there are, as we have seen, 32 premaiotic chromosomes, and the synaptic loops of the first maiotic division resolved themselves into 16 gemini, so that we are led to conclude that gemini consist each of two somatic chromosomes joined end to end." Neither have we found a condition which could certainly be interpreted as parasynapsis. The condition illustrated in Fig. 3 might be interpreted as parasynapsis, but could as readily be considered as a precocious splitting of the chromosomes in tetrad formation. The fact that there are four U-shaped primary spermatocyte chromosomes and that there are eight similar shaped spermatogonial chromosomes would be evidence in favor of parasynapsis. There is no genetic evidence in favor of parasynapsis.

SUMMARY.

1. The actively dividing cells are not distributed throughout the whole lining of the seminiferous tubules but are limited to elliptical areas. 2. These areas never exceed two thirds the circumference of the tubules and sometimes consist of only a few cells. 3. The greatest diameter of the ellipse is always lengthwise of the tubule. 4. The areas of actively dividing cells are more numerous and larger in the superactive male. 5. There are thirty-eight spermatogonial chromosomes, which according to size and shape may be grouped into eighteen similar pairs and a pair which is unequal in size which we have designated as the XY-pair. 6. Both the primary and secondary spermatocyte cells have nineteen chromosomes. 7. The XY-tetrad is precocious in its division and the resultant chromosomes are unequal in size. 8. All the chromosomes divide equally in the second division. 9. There is some evidence of parasynapsis.

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EXPLANATION OF PLATES.

All the drawings were made with the aid of a camera lucida, a 1.9 oil immersion objective and a number 10 ocular at table level. Then they were enlarged two diameters. The reproductions were reduced $\frac{1}{3}$ from the original.

Plate I.

Fig. 1. Spireme of spermatogonial division.

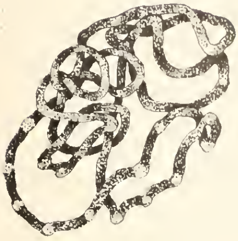
Fig. 2. Metaphase plate polar view of spermatogonial division showing nineteen pairs of chromosomes. The pair numbered 19 is the XY-pair.

Fig. 3. Formation of the primary spermatocyte chromosomes.

Fig. 4. Metaphase plate of primary spermatocyte chromosome showing the unequal XY-tetrad.

Fig. 5. Formation of primary spermatocyte chromosomes. This is a later stage than Fig. 3.

Fig. 6. Metaphase plate of primary spermatocyte chromosomes.



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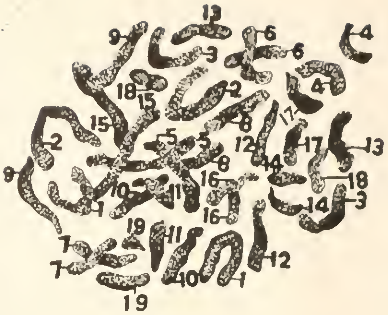
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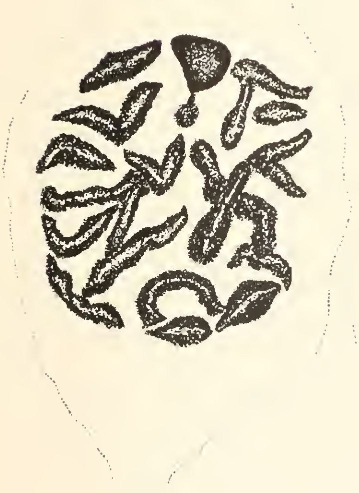

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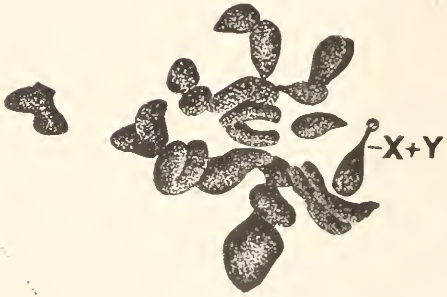

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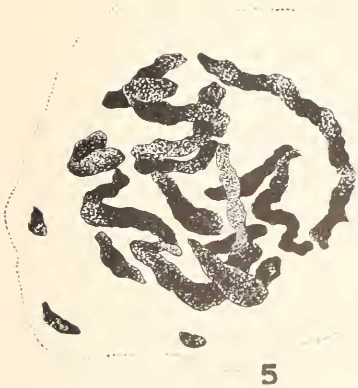

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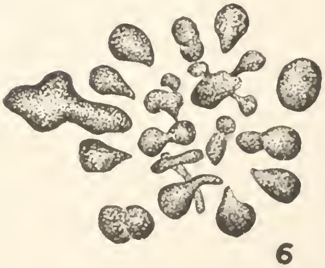

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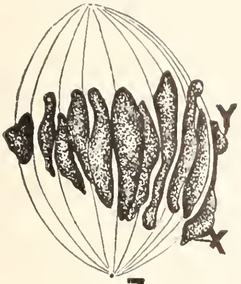
PLATE II.

FIG. 7. Metaphase plate lateral view of primary spermatocyte showing the precocious and unequal division of the XY-tetrad.

FIG. 8. Same as 7, except the XY has entirely divided.

FIGS. 9 AND 10. Anaphase primary spermatocyte.

FIGS. 11 AND 12. Metaphase plate polar view of secondary spermatocyte divisions.



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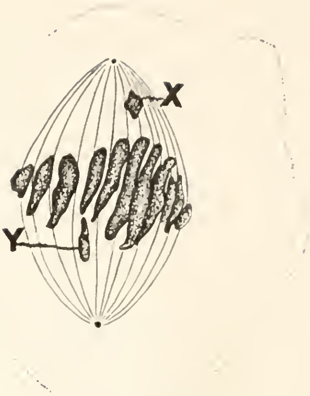

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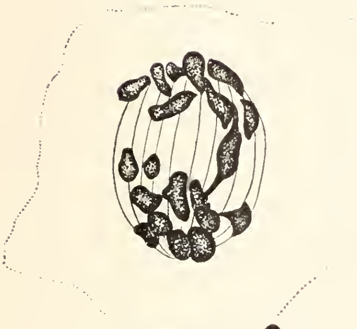

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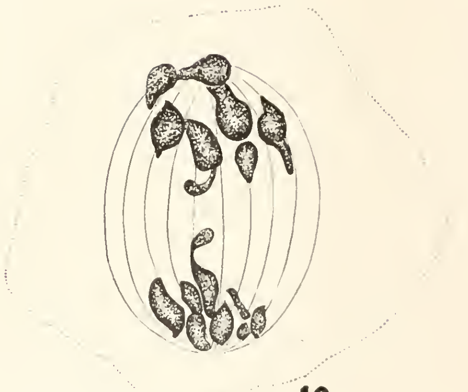

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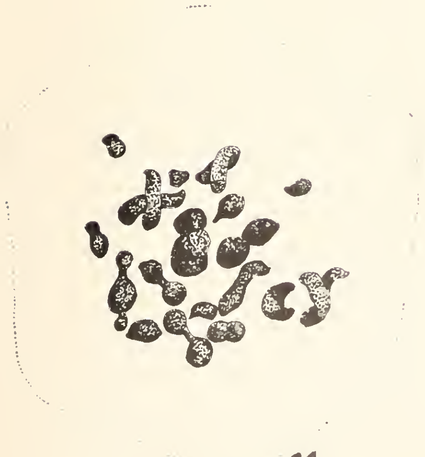

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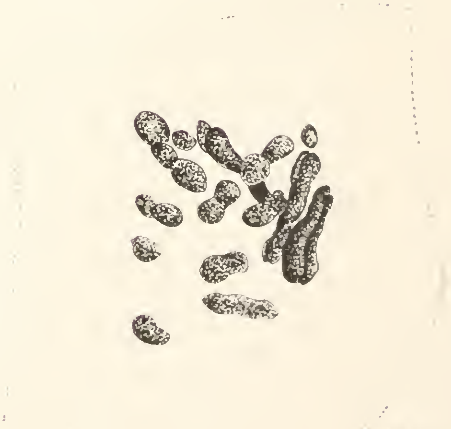

12

ON THE INHIBITION OF ANIMAL LUMINESCENCE BY LIGHT.

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Since Allman's (1862) observations on Ctenophores, many researches have left no doubt of the inhibition of the luminescence of these forms by light. There is not only inhibition of the irritability but an actual disappearance of photogenic substance, which will return again in the dark, even in extracts of Ctenophores, provided the photogenic cells have not been entirely broken up. But a centrifuged extract of Ctenophores exposed to sunlight shows only slight recovery of its power to luminesce in the dark, as compared with a control kept continually in the dark. The centrifuged extract contains photogenic granules but not photogenic cells, and I think it doubtful if isolated granules recover the power to luminesce, although this point is difficult to ascertain with certainty.

Heinemann (1872) observed inhibition of the luminescence of *Pyrophorus*, the West Indian elaterid, and I have observed marked inhibition of the luminous extract of *Cypridina* (Harvey, 1925) but none of the whole living animals themselves.

Massart (1893) found a day and night periodicity of luminescence in *Noctiluca*, Zacharias (1905) in *Ceratium*, B. Moore (1908) in some animals of the plankton, presumably copepods, and Heymans and A. R. Moore (1923) in *Pelagia noctiluca*, a medusa. It is reported that these forms will not luminesce in the daytime even when they have been kept in a dark room. In a recent note A. R. Moore (1926) was unable to confirm the observations of Heymans and Moore in *Pelagia* and I have also been unable to observe any inhibiting effect of strong sunlight on either the local or general luminescence of this form, although the exposure was from sunrise until noon of a cloudless day in December. Parker (1920) finds inhibition of luminescence of the pennatulid, *Renilla*, by sunlight.

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[1] Appointed to the A. A. A. S. table at Naples, Oct. 1925–Feb. 1926.

During the past winter at Naples I had an opportunity of examining many luminous forms after exposure to light and these observations, together with previous ones, are collected in this paper. As sources of light the sun (between 10 A.M. and noon), a large carbon arc and a small carbon arc were used. All the light passed through glass and water to absorb the heat. Light from the carbon arcs was condensed by glass lenses. The large arc gave (judging by its inhibition of *Cypridina* luminescence) at least 10,000 foot-candles while the smaller one, perhaps half of that. Either the whole animal or extracts of luminous material were exposed for the times indicated.

As to periodicity of luminescence, I have never yet observed an animal which would not luminesce during the daytime, provided it had been kept in the dark for a few hours previously. I have never studied the dinoflagellates but the copepods at Naples (Dec., Jan.), *Noctiluca miliaris* at Misaki, Japan (July), and Friday Harbor, Washington (Aug.), *Balanoglossus minutus* at Naples (Dec.), *Ptychodera* sp. at Bermuda (Dec.), and *Pelagia noctiluca* at Naples (Dec.) luminesce during the day.

Other forms in which the *whole animal* has been exposed to sunlight for the time indicated, and shows no inhibition of luminescence when tested immediately in a dark room are as follows: the ophiurian, *Amphiura squamata* (Naples, Dec., 15 min.); the annelids, *Chætopterus variopedatus* (Woods Hole, July) and *Microscolex phosphorea* (Naples, Dec., 20 min.); the radiolaria, *Thalassicolla nucleata*, *Colozoun inerme* and *Sphærozoön punctatum* (Naples, Dec. 15 min.); luminous bacteria; luminous fungus (*Panus stipticus*) (Woods Hole, Aug.); pennatulids, *Pennatula phosphorea* (Naples, Dec.), *Cavernularia haberi* (Misaki, Japan, July), *Ptylosarcus* sp. (Friday Harbor, Wash., July); *Pyrosoma* sp. (Monaco, Jan., 60 min.); *Cypridina hilgendorfii* (Misaki, Japan, July); the medusæ, *Mitrocoma cellularia*, *Phialidium gregarium*, *Stomatoca atra*, and *Æquora forskalia* (Friday Harbor, Wash., Aug.); the squid, *Watasenia scintillans* (Japan, May); the fish, *Monocentris japonica;* and the fire-flies, *Luciola parva* and *viticollis* (Japan, July).

Forms in which a luminous extract has been exposed to the carbon arc and in which no inhibition is to be noted are: *Pholas* *dactylus* (large arc, 4 min.); *Heteroteuthis dispar* (small arc, 3 min.).

As to inhibition of fireflies, I have observed no inhibition of the luminous organ of *Photuris pennsylvanica* removed from the body and mashed on a slide (so that it luminesced continuously) after 4 minutes' exposure to 15,000 foot-candles. However, when the luminous organ (alone) in the intact animal is directly exposed to the above light, the normal flashes of the animal as well as the flashes from electrical stimulation (of the thorax) are inhibited and remain so for some seconds after the light is screened. Illumination of the eyes by 15,000 foot-candles while keeping the luminous organ in the dark does not inhibit the flashing.

Pyrophorus noctilucus sent to Woods Hole from Cuba shows no inhibition of the teased organ in 15,000 foot-candles illumination but if one of the two thoracic organs is exposed to sunlight or the carbon arc light (passing through water) for two minutes, while it is glowing steadily, it will be observed that this exposed organ now glows less strongly than the other in the dark.

We are evidently dealing in these cases with no true inhibition of luminescence of luminous material but with an effect of light on the local nerve mechanism of stimulation in the organ itself, a process quite different from that in *Cypridina*.

It will thus be observed that inhibition of luminescence is rather rare and that Ctenophores and *Cypridina* remain the best known cases. *Noctiluca* and the Dinoflagellates need reinvestigation and it is possible that some species of copepods show inhibition. A large number of other forms do not.

In *Cypridina* the inhibition is due to acceleration of the spontaneous (without luminescence) oxidation of luciferin. Light has no inhibiting effect in absence of oxygen. This probably explains why the whole *Cypridinas* are not inhibited, for the great mass of luciferin must be kept under anaërobic conditions in the gland.

I have also investigated the Ctenophores to determine if their luminescence was inhibited by light in absence of oxygen and was surprised to find inhibition by light when the last traces of oxygen were removed from a *Beroë, Eucharis* or *Mnemiopsis* extract, with platinized asbestos and hydrogen. I later discovered that Ctenophore extracts will luminesce in complete absence of oxygen[1] and presume that oxygen must be bound in some way with the photogenic granules. It is quite possible, then, that the inhibition by light in Ctenophores may also be due to photochemical acceleration of oxidation (without luminescence) of photo-genic material.

Conclusions.

Inhibition of luminescence of photogenic material by light is not a general phenomenon. Ctenophores remain the best known case, but in my experience pennatulids and medusæ show no inhibition. *Cypridina* extracts are also inhibited if they contain oxygen and the inhibition seems to consist of an oxidative destruction of photogenic substance.

I have never yet observed an animal that would fail to luminesce in the daytime provided it had been kept in darkness for a few hours previously. Flagellates and copepods should be studied further to test the question of day-night rhythm of luminescence.

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OXYGEN AND LUMINESCENCE, WITH A DESCRIPTION OF METHODS FOR REMOVING OXYGEN FROM CELLS AND FLUIDS.

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(From the Stazione Zoölogica, Naples.)

So many luminous animals require dissolved gaseous oxygen for luminescence that one might expect all to need it, but I have recently discovered certain forms which can luminesce without oxygen. Perhaps they utilize oxygen already bound up with the luminous material or perhaps the old question as to the storing of oxygen in cells must be reopened again. One should like to say, "Without oxygen neither life nor light." Such an epigram can only be true when, by oxygen, we refer to the element both free and combined. If we speak of anaërobic existence, we must also recognize anaërobic luminescence.

I arrived at this discovery through the observation that, although the luminescence of *Cypridina* is inhibited by strong light in presence of oxygen but not in its absence, the luminescence of *Mnemiopsis* and other Ctenophores is inhibited by light whether oxygen be present or not. This led me to reinvestigate methods of removing oxygen from fluids and the discovery that Ctenophore extracts can still luminesce under anaërobic conditions where other luminous forms (including *Cypridina*) show no trace of luminescence. It seems very probable, then, that in Ctenophores oxygen is firmly bound and that this is the reason we observe inhibition of their luminescence by light in absence (apparent) of oxygen. If oxygen is not bound in Ctenophores, their luminescence must result from a totally different type of reaction from that in other forms, for I believe the means employed for oxygen removal are without criticism.

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[1] Appointed to the A. A. A. S. table at Naples, Oct. 1925–Jan. 1926.

There are four convenient methods of removing the last trace of oxygen from biological fluids. I say methods for *biological* fluids because none of these methods involves very great changes in H-ion concentration that might bring about changes in the fluid, although all of them are not suited for living cells. All these methods prevent the luminescence of bacteria (Harvey and Morrison, 1923) and *Cypridina*, which certainly means less oxygen than 10^{-5} atmospheres, and most of them involve nascent hydrogen.

1. By addition of cells actively using oxygen such as muscle, yeast or bacteria. If the fluid is a cell extract requiring oxygen, it will use up its own oxygen on standing in a narrow tube. The reducing power of cells varies but practically all will reduce methylene blue and most will reduce indigo-carmine. For the significance of this in terms of oxidation-reduction potential the reader is referred to the papers of Clark (1923–25).

2. Nascent hydrogen can be generated in the fluid by adding aluminium amalgam or magnesium plus some neutral ammonium salt. In the first case $Al(OH)_3$ is formed and in the second an ammonium-magnesium salt. In either case the P_H does not change much, and the oxygen is quickly washed out.

3. Small amounts of sodium hydrosulphite (or hyposulphite, $Na_2S_2O_4$, not thiosulphite) may be added to the fluid. It is best to dissolve the hydrosulphite in a powerful buffer and to buffer the fluid under investigation as the hydrosulphite is acid. To determine when the right amount of hydrosulphite has been added methylene blue can be used as indicator. It is decolorized when reduced by the hydrosulphite, which also absorbs all oxygen in the solution. Shaking with air or addition of potassium ferricyanide [$K_3Fe(CN)_6$] will oxidize the hydrosulphite with return of the blue color to methylene blue. The advantage of hydrosulphite is its practically instantaneous production of anaërobic conditions. The disadvantage is a possible injurious effect on living cells, which I believe should be investigated rather carefully. My own observations show that the ciliated cells of the gills of *Mytilus*, lightly stained in methylene blue, stop beating and the blue color disappears (reduction) in hydrosulphite sea water very quickly. When fresh aërated sea water

is added the blue color returns and then the cilia begin beating again.

The luminescence of a mixture of *Cypridina* luciferin and luciferase is immediately extinguished by hydrosulphite and returns again on shaking with air at a time when methylene blue is just beginning to show a slight blue color. If some dry powdered *Cypridinas* are added to hydrosulphite sea water, it will be observed that fragments of undissolved luminous material are luminescent but the light disappears very quickly. There is apparently some air entangled, possibly mechanically, with the dry photogenic substances and when this is used up luminescence ceases. I had previously made this observation in another manner (Harvey, 1920).

In using methylene blue as an indicator, it must be borne in mind that mere removal of oxygen will not reduce methylene blue but that once reduced, a blue color will return when traces of oxygen are present. However, the reoxidation of methylene blue is rather slow except in alkaline solutions. The color change of methylene blue is also affected by light (Clark, 1925). Absence of luminescence in *Cypridina* luciferin-luciferase or in luminous bacteria can also be used as an indicator of lack of oxygen (the former is more delicate; bacteria begin to luminesce at an oxygen pressure = .0053 mm. Hg) and mere removal of oxygen is sufficient for cessation of luminescence in both cases.

4. Addition of platinized asbestos to the fluid and passage of hydrogen through it. This affords a quite rapid method of removing oxygen from fluids and the hydrogen need not be absolutely free of oxygen. It is far more rapid than bubbling pure hydrogen through the fluid without platinized asbestos. By pure hydrogen I mean hydrogen that has been passed over platinized asbestos heated to dull redness in a quartz tube. Such hydrogen contains oxygen in equilibrium at 10^{-26} atmospheres. It must not come in contact with rubber or oxygen will be absorbed from the rubber in sufficient amount to cause luminescence of *Cypridina* luciferin-luciferase solution. My studies on luminescent solutions have impressed me with the difficulty of removing small amounts of oxygen from fluids and the slowness with which a fluid comes into equilibrium with a

gas. I believe the addition of platinized asbestos to the fluid through which hydrogen is passed will prove of great value to biologists in the study of cells under anaërobic conditions.

Instead of Pt asbestos, colloidal Pt or colloidal Pd may be used. The latter is conveniently prepared by adding to 5 cc. 0.2 per cent. $PdCl_2$ just enough dilute NaOH to make the $PdCl_2$ darken without giving a precipitate of basic $PdCl_2$, and then one drop of .042 per cent. hydrazine hydrate. The finely divided Pd adsorbs a good deal of hydrogen and will keep a solution free of oxygen for some time, as can be observed by using as indicator the luminescence of a mixture of luciferin and luciferase.

In order to show that the luminescence of Ctenophores, like *Beroë*, is independent of dissolved oxygen, it must be recalled that an extract made by squeezing *Beroë* through muslin and filtering through filter paper is non-luminescent itself, but gives a brilliant light on addition of fresh water, *dilute* salt solution, or various cytolytic substances like saponin, sodium glycocholate, ether, etc. Accordingly I tried the experiment of mixing *Beroë* extract made free of oxygen by $Na_2S_2O_4$ with $m/10$ Na_2HPO_4 solution, also made free of oxygen by $Na_2S_2O_4$. The Na_2HPO_4 was used instead of fresh water in order that the reaction would be alkaline enough to allow rapid coloration of leuco-methylene blue, added as an indicator, should any oxygen leak into the apparatus. This is shown in Fig. 1. In *A* is placed *Beroë* extract + $Na_2S_2O_4$

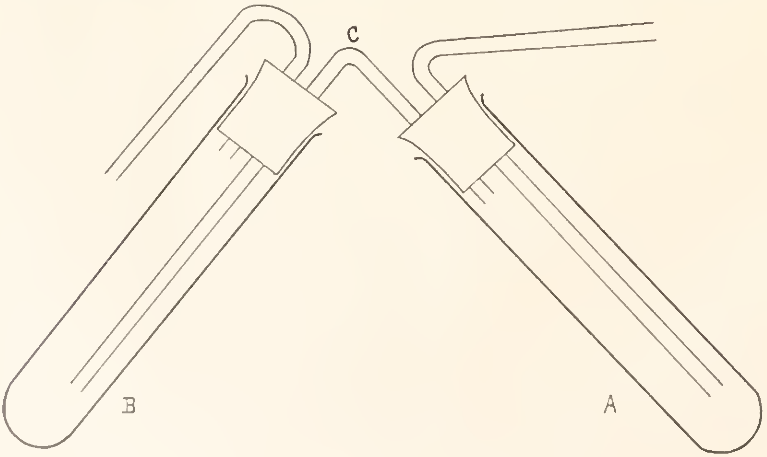

FIG. 1.

and in B $m/10$ Na_2HPO_4 solution ($P_H = 9$) + $Na_2S_2O_4$. The tubes allow a flow of pure hydrogen through a lead tube to wash out the air, and the only rubber parts are the stoppers of tubes A and B. In such an apparatus the contents of A and B can be mixed in hydrogen without the slightest blueing of leucomethylene blue and oxygen-free *Cypridina* luciferin can be mixed with oxygen-free luciferase (by methods 3 or 4) without any luminescence appearing. However, when the oxygen-free *Beroë* extract (by methods 3 or 4) is mixed with the oxygen-free $m/10$ Na_2HPO_4 solution, just as bright a luminescence occurs, as in the control tests when mixed in presence of oxygen.

The experiment has been varied by using Pt asbestos in the *Beroë* extract and in the water and passing pure hydrogen through the system for an hour (although methylene blue can be decolorized in two or three minutes by the same flow of hydrogen), but a bright luminescence always results on mixing the *Beroë* extract with the water in the hydrogen atmosphere. When air is admitted and the fluid shaken, no further luminescence is to be observed.

The experiment has been repeated so often that I feel certain that Beroë extract will luminesce in absence of dissolved oxygen. *Eucharis multicornis*, another Ctenophore, behaves as *Beroë*, as does also the medusa, *Pelagia noctiluca*. *Pennatula phosphorea* extract, on the other hand, requires oxygen, as I had previously observed for the pennatulid, *Cavernularia haberi* Harvey (1917). A *Pennatula* extract in sea water mixed with fresh water in absence of oxygen[1] gives no light but when shaken with air the mixture luminesces faintly. It is somewhat surprising that the pennatulids should differ in this respect from the Ctenophores and *Pelagia* as the general aspects of the luminescence in these forms is similar.

None of these animals give the luciferin-luciferase reaction. The luminescence seems to be connected with the dissolution of granules. In the luminous slime of *Pelagia*, which sticks to the finger when rubbed over the exumbrella, can be observed with the microscope, cells densely crowded with granules somewhat less than 1 μ in diameter. These granules and some cell fragments will pass filter paper and centrifuging such a filtrate will throw down the cell fragments and many granules, leaving suspended only granules. When added to fresh water this centrifuged material gives a bright luminescence (because many granules are thrown down) while the suspension above gives a fainter luminescence. I could observe no cells in the suspension but when mixed with fresh water in complete absence of dissolved oxygen (method No. 4, hydrogen passed 20 mm.), as bright a luminescence occurred as when oxygen was present. I therefore believe that the oxygen is bound in these photogenic granules and that luminescence occurs upon their dissolution. That just enough or more than enough oxygen is so bound would be indicated by the fact that opening the tube containing the mixed fluids to the air results in no further luminescence.

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[1] Mixing in air of course results in luminescence.

This same experiment has been repeated with filtered and centrifuged *Beroë* extract, with the same result, luminescence of granular material in absence of oxygen.

The Radiolaria also require no free oxygen for luminescence. *Thalassicolla nucleata* and also colonies of *Colozoun inerme* mixed with platinized asbestos in sea water through which pure hydrogen is passed for 45 minutes will still luminesce whenever shaken. A colony of *Colozoun inerme* was broken up into separate cells (but these left uninjured) mixed with platinized asbestos and pure hydrogen passed through it for 20 minutes. It was then mixed in a pure hydrogen atmosphere with water and platinized asbestos through which pure hydrogen was passed for 20 minutes. A bright "starry" luminescence resulted, similar to that of *Beroë* extract when mixed with water.

After these rather startling (to me) results I thought it worth while to reinvestigate the necessity of oxygen for luminescence in other animals, noting carefully if the lack of oxygen caused luminescence to disappear when the whole animal was tested and when its secretion was tested. For we are especially interested in knowing whether the luminescence of dissolved or finely divided photogenic material is stopped by lack of oxygen rather than whether intact cells cease to luminesce in absence of oxygen. It is of course perfectly well known that luminous bacteria cease to luminesce in absence of oxygen but one could not make the same statement of the *extract* of luminous bacteria for luminous material cannot be obtained apart from the living bacterial cell. The same is probably true of luminous fungi. They (*Panus stipticus*) require oxygen for luminescence. If ground in a mortar, *Panus stipticus* luminescence ceases almost immediately, so that an extract cannot be obtained.

The work of many investigators has shown that luminous *extracts* of *Pholas* (lamellibranch mollusc), *Cypridina* (ostracod crustacean), *fireflies*, *Cavernularia* (pennatulid), *Photoblepharon*,[1] *Anomalops*,[1] and *Malacocephalus* (fish) require oxygen for luminescence. I have recently investigated the following forms using extracts of the animals whenever possible.

Microscolex phosphorea, an earthworm producing a slime on stimulation, brightly luminescent with a yellowish light. The worm can be dried over $CaCl_2$ and gives a bright light on moistening with water. Worms shaken in pure hydrogen give no luminescence but produce a slime which luminesces immediately when air is admitted.

Chætopterus variopedatus, a polychæte producing a slime with bluish light on stimulation. In sea water the luminescence of the slime lasts long enough for one to observe the disappearance of luminescence when pure hydrogen is bubbled through it and the reappearance on admitting air.

Acholoë astericola, a polynoid worm whose scales emit a bright yellowish luminescence on stimulation. The light comes from groups of gland cells under the cuticula ending in a papilla with a pore, but not enough secretion is extruded to form a luminous solution. The whole animal when slowly heated in sea water or shaken gives a bright luminescence (over the scales) but if slowly heated or shaken in sea water with platinized asbestos through which hydrogen is passed, there is no luminescence.

Thelepus cincinnatus, a polychæte allied to *Polycirrus*, produces a luminescent slime on handling. When the whole worms are placed in a tube through which pure hydrogen is bubbled the luminescence disappears, but returns again when air is admitted.

Amphiura squamata, a brittle star whose arms produce a yellowish luminescence. The individual gland cells lie beneath the surface of certain plates and possess ducts that empty through pores in the cuticula, but not enough secretion is passed to the outside to give a luminous solution. The whole animals stimulated electrically in sea water plus platinized asbestos and air give a bright luminescence but no luminescence when stimulated in sea water plus platinized asbestos through which hydrogen has been passed for some time.

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[1] The light of these forms is due to symbiotic luminous bacteria living in the organ.

Balanoglossus minutus.—The whole of the skin produces a luminescent slime whose light does not last very long. If the whole animal is shaken in sea water through which pure H_2 is passing, there is no luminescence but on admitting air both worm and sea water show luminescence.

Copepods.—(Several species.) The cells are unicellular glands in definite positions in the body or in the legs, secreting to the exterior. When heated slowly in sea water with air the copepods give a bright luminescence but when heated slowly in sea water and platinized asbestos through which hydrogen was passed there was no luminescence. In one experiment the temperature was raised to boiling in the hydrogen atmosphere, then cooled and air admitted. The *solution* became faintly luminescent. A small amount of oxygen will cause luminescence and the hydrogen must be passed for some time to obtain anaërobic conditions.

Heteroteuthis dispar.—A squid in which a secretion from a gland (part of ink sac) is shot into the sea water as a bluish luminescent cloud of small granules often sticking together in clumps and filaments. On a microscope slide under a cover slip the secretion luminesces only at the edges in contact with air (but luminesces uniformly when the cover slip is lifted). When hydrosulphide is added to the secretion in a test tube the luminescence disappears except at surface but returns on shaking with air. Oxygen is necessary for luminescence.

Quatrefages observed luminescence of *Noctiluca* under what he regarded as anaërobic conditions whereas E. B. Harvey (1917) found the luminescence of *Noctiluca* to become very faint in a current of impure hydrogen (made in a Kipp generator but last traces of oxygen not removed) but to regain its brightness when air was admitted. It would seem that *Noctiluca* must require oxygen for luminescence. In view of my experiments with

Radiolaria and Cœlenterates it would certainly be worth while to test the luminescence of dinoflagellates, hydroids, siphonophoræ and medusæ in absence of oxygen. Many other higher forms await careful experimental investigation.

SUMMARY.

Simple methods for completely removing oxygen from biological fluids are described and indicators for absence of oxygen discussed.

Most luminous animals require free gaseous dissolved oxygen for luminescence but a few can luminesce without such oxygen. These are the Ctenophores; the medusa, *Pelagia noctiluca;* and Radiolarians. Pennatulids require oxygen as do all annelida, ophiurians, cephalopoda, copepoda, and balanoglossids tested.

It is found that in *Beroë* and *Pelagia* the photogenic granules (without cells) luminesce in absence of oxygen, and it is suggested that the proper amount of oxygen for luminescence is bound up in the photogenic granule, and cannot be removed by the drastic methods of oxygen-removal herein described.

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COMPLETE SEX-REVERSAL IN THE VIVIPAROUS TELEOST *XIPHOPHORUS HELLERI.*

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I. Introduction.

During the progress of the study on sex-differentiation of *Xiphophorus helleri* it became apparent that sex was not a fixed factor in this species. It was found (Essenberg, 1923) that 50 per cent. of the young females transformed to males before sexual maturity was reached. There were also indications that sex-reversal is not confined to immature fishes but that it may occur in adult specimens which have previously born young. To test the merits of such indications experimental plans were conceived which, together with the results, are considered in the present paper.

With the most favorable environment which prevailed at the Hull Zoölogical Laboratory and the abundance of *Xiphophorus helleri*, it was relatively an easy matter to initiate experiments. A large number of adult females were isolated into separate aquaria for observation. Close watch of these specimens was kept for more than a year but with totally negative results.

In the fall of 1922, the writer became associated with the Medical School of the University of Missouri, where the work was to be continued. Early in the spring of the following year a shipment of fish was received from the University of Chicago, for which I wish to express my gratitude to Dr. A. W. Bellamy, and another shipment was procured from the Crescent Fish Farm of New Orleans. Fishes of both shipments were mixed before they were isolated into separate aquaria. Strict records of each isolated fish were kept. The records were to show the date of isolation, date of birth of young, and the number of young at each birth.

At the beginning of July, 1923, one of the adult females which had given birth to forty young on the second of June and fifty-three on the fourth of May failed to bring forth young. It is necessary to state here that the species under consideration reproduces monthly with almost chronological regularity. At the time mentioned there were no definite signs of sex-reversal and it was thought that owing to some change in environment, birth of young was delayed. A month later, that is the beginning of August, signs were available as to what was going on with female B16. September the 12th, when the writer left for Norman, Oklahoma, to occupy his present position, the fish had reached advanced stages of sex-reversal.

Owing to the courtesy of Dr. G. W. Tannreuther of the Department of Zoölogy, to whom I wish to express my indebtedness, my research material reached Norman in good shape a few weeks later. Female B16 was an unmistakable male except body form by the first of December and was mated to a virgin female. On February 25, 1924, this pair gave birth to eight young, all of which were to all appearances normal. Two weeks later five of the young fish died owing to a sudden drop of temperature. The remaining three reached sexual maturity with one male and two females as a sex-ratio.

The stock was greatly increased in the spring of 1924 when additional material was procured from the Crescent Fish Farm. A larger number than ever before of adult females, not less than three years of age, were isolated for observation. Of this group three females transformed to males before the end of the year. One, female C32, transformed without previously giving birth to young and for this reason is dismissed from further consideration in this study. Female C3 gave birth to one litter and female C14 three litters of young before sex-reversal took place. Both fishes were mated to virgin females, but young were obtained only from the mating in which C3 was the male. From this mating five young were obtained, all of which reached sexual maturity. The sex-ratio was two males to three females.

As stated above, in the mating in which C14 was the male no young were obtained. The mating was repeated with other virgin females but without success. Upon killing, the autopsy revealed malformation of the sperm duct which was obstructed at its anterior end. The testes were well formed and contained abundant spermatophores.

For convenience of description the process of sex-reversal in *Xiphophorus helleri* may be divided into three, more or less, definite stages. The first ranges from a normal female to the enlargement of the anal fin, the second includes the formation of the gonopod, and the third is concerned with the development of the "sword" in the tail fin.

II. First Stage in Sex-reversal.

The first indication of sex-reversal in these fishes is the absence of the regular monthly delivery of young. To be sure, there are other factors which inhibit reproduction such as lack of fertilization, certain temperatures, light, and composition of the water, but these are exceptions and can be eliminated in normal and well-balanced aquaria. With the failure of reproduction the observer's attention is focused on other landmarks in the history of sex-reversal, the most important of which is a characteristic black area known as the puberty spot.

The puberty spot is found in all sexually mature females and is located just above the pelvic fin. Its size varies with the age of the fish or rather with the size of the ovary. In young virgin females it is less than one fourth of an inch in diameter, whereas in females of three years of age it approaches half an inch in diameter. The puberty spot is caused by the black pigment visible through the body wall which accumulates in the peritoneum adjacent to the ovary. With onset of sex-reversal the black pigment in the peritoneum gradually fades and in the course of six to eight weeks the puberty spot has entirely disappeared.

Sex colorations in *Xiphophorus helleri* exhibit very little, if any, dimorphism. During sex-reversal the bright red and black pigments of the fins and lateral line dim to such an extent that the fish becomes, so to speak, "invisible." This appearance of the animal is retained until the gonad of the opposite sex has begun to grow and is capable of producing a certain amount of the male sex hormone.

The internal changes which take place during this stage are significant and must, to a certain extent, be reviewed here. For further details of description and figures on sex-differentiation the reader is referred to Essenberg (1923).

As far as the writer knows, the causative factor or factors in sex-reversal have not been successfully demonstrated. That these factors reside in the gonad and possibly in the germ cells themselves has been substantiated beyond a doubt by experimental data. Whenever those factors become functional the entire ovary is subject to hopeless degeneration. At first the large ovocytes disintegrate, then the medium-sized ones, and finally the young ovocytes which are found close to the epithelium of the ovarian cavity follow their two predecessors. Also the epithelium covering the ovary, which originates from the peritoneum of the body cavity, disintegrates completely. The epithelium of the ovarian cavity, however, is not subject to disintegration. It shrinks and assumes more or less a tubular form, its cells, judging from their appearance, undoubtedly undergo retrogressive changes but no disintegration is noticeable. After a period of rest this epithelium becomes active and proliferates cells which go to form the gonad of the opposite sex—the testis. The process of testicular formation which belongs to the present stage of sex-reversal includes the proliferation of germ cells from the remains of the epithelium of the ovarian cavity and the formation of sex-cords. It includes two stages of sex-differentiation called by the writer "Early and Middle Stages of Tubule Formation" (Essenberg, 1923, pp. 57–58).

It is interesting to note that the epithelium of the ovarian cavity which gives rise to the definitive male cells originates, as does the epithelium covering the ovary, from the peritoneum of the body cavity.

While the process of disintegration is taking place, the vitality of the transforming animal is reduced to the minimum. Voluntary motion which is so characteristic of *Xiphophorus helleri* is seldom noticed; the fish rests most of the time and moves only to escape danger or because of hunger. As to the latter it may be said that the animal takes very little food of any kind during the process of ovarian disintegration. This is a critical stage to the animal. The least change in the animal's environment, such as slight fluctuation in temperature, water composition or disturbance will inevitably result in the death of the fish. It seems probable that sex-reversal in *Xiphophorus helleri* is not preceded by the increase of metabolic rate but that the opposite is true. Nor is it true that the transformation is caused by tuberculosis or any other kind of disease as far as it can be detected either macro- or microscopically.

III. Second Stage in Sex-reversal.

The second stage in sex-reversal deals with the transformation of the anal fin of the female into a functional intromittent organ or gonopod and with the parallel development of the gonad.

The anal fin of the female consists of ten pairs of rays all of which are approximately of the same length and diameter. The above statement applies equally well to the anal fin of young fishes during the indifferent stage of development. During sex-differentiation in the male direction or sex-reversal, which in *Xiphophorus helleri* is always from female to male, several pairs of the rays undergo a peculiar metamorphosis to form a copulatory organ. The first sign of such transformation is the thickening of the third pair of rays. While this is in progress, the third, fourth, and fifth pairs of rays elongate until approximately twice the length of the original fin is reached. The first, second, and sixth to tenth pairs of rays are subject to no special changes and remain rudimentary. The third pair reaches a thickness several times its original diameter and serves as a supporting bar or rod of the gonopod. The apex of this pair of rays together with the fourth and fifth pairs form hooks and counterhooks which serve the purpose of anchorage to the female genital pore during copulation. It is not a hollow penis-like structure but a solid bar which acts as a guide in transmitting of spermatophores from male to female genitals.

The development of the gonad during the second stage of sex-reversal corresponds to the "Late Stage of Tubular Formation" in sex-differentiation. The solid sex-cords which were formed during the first stage acquire a lumen and become branched and subbranched. The cells constituting the sex-cords or tubules, which thus far were not unlike their sister cells of the peritoneum undergo characteristic transformation. The cytoplasm as well as the nucleus increases in size and assumes a spherical form. When these cells have completed their differentiation they can not be distinguished microscopically from the primordial germ cells of the indifferent stage.

IV. The Third Stage in Sex-Reversal.

The third stage in sex-reversal is characterized externally by the development of a "sword"-like process in the tail fin and internally by spermatogenesis.

It is true that there is a slight overlapping between the second and third stages, yet it is desirable to consider these separately for the reason that they constitute definite landmarks in the process of sex-reversal.

The sword of the caudal fin begins to develop slightly before the gonopod reaches its completion. It is formed from the ventral lobe of the tail fin. The ten ventral rays of this fin are involved. Most of the elongation falls upon the sixth to the tenth and the utmost length is reached by the eighth ray. The length of the fully formed sword approximates the total length of the fish. It is abundantly supplied with brilliant colors and its sole purpose seems to be display.

The development of the testis in this stage of sex-reversal consists of spermatogenesis. A number of germ cells develop in a constricted portion of the sex-cord or tubule and form an acinus or spermatocyst. Every cell in such a cyst is in the same stage of development from the beginning to the end and they ultimately form the spermatophore. At the end of the so-called "Middle Stage of Tubular Formation," the germ cells are primary spermatocytes. The secondary spermatocyte stage is reached by cell division which doubles the number of germ cells and the volume of the acinus. With another cell division which doubles the cell number but not volume of the acinus the germ cells reach the spermatid stage. With the metamorphosis of spermatids into spermatozoa the acinus which thus far has been so tightly packed with germ cells that it simulates a syncytium now becomes luminated. The lumination owes its origin to the fact that the spermatozoa move towards the periphery of the acinus and form a lining to the thin membranous wall of the acinus. The lining consists of only one layer and is formed by the nuclei or heads of the spermatazoa. The tails project freely into the lumen. With this the acinus has metamorphosed into the mature spermatophore which during copulation reaches the female genitals by the aid of the gonopod.

V. Discussion.

Fertility before Sex-Reversal.

To eliminate the chance of error and prevent misunderstanding, it was decided that only those females which are definitely known to have produced young will be considered in the study of sex-reversal. The records of female B16 shows that she gave birth to 53 young on the 4th of May, and to 40 young on June the 2d, 1923. The records of female C3, which are equally complete, show that only one litter of young were born before she began to change in the male direction. If it is remembered that the females of *Xiphophorus helleri* reach sexual maturity before they are one year of age, and that the fishes were at least three years of age before they came under the writer's observation, it is seen that females B16 and C3 have been experiencing the instincts of motherhood not less than two years before they experienced the instincts of fatherhood.

Fertility after Sex-Reversal.

From the mating in which B16 was the male, eight young were born. C3 became the father of five young, all of which developed to normal, sexually mature fish. It can be stated that the mortality in the progeny of B16 was wholly due to the inability to control temperature in the aquaria. The sex ratio of the young obtained compares favorably with sex ratios found by the writer in his study of sex-differentiation (1923).

The Origin of Testicular Tissues.

It has been shown (Essenberg, 1923) that the definitive germ cells of *Xiphophorus helleri* are not linear descendants of primordial germ cells but originate from peritoneal epithelium. The primordial germ cells either entirely disintegrate (females) or become segregated and thus never take part in definitive germ cell production (males). The epithelium of the ovarian cavity which gives rise to definitive ovocytes proliferates cells which go to form the testes of the transforming fish. The epithelium of the ovarian cavity originates in the following fashion. As the primordial germ cells of this teleost project into the body cavity, they are covered by the peritoneal epithelium. During the indifferent stage the gonads are paired. If the young fish develop in the male direction, the paired or bilateral relation is retained permanently. In case the animal develops into a female, the undifferentiated bilateral gonads fuse medially in such a manner that the medial parts of the covering of the gonads form the epithelium of the ovarian cavity. In the course of sex-reversal the entire ovary disintegrates except the epithelium of the ovarian cavity. From it cells proliferate which form sex cords and later seminiferous tubules.

Proof to the effect that the same is true in birds was furnished by the excellent work of Fell (1923). This author had the unique opportunity to study the gonads of eight arrhenoid birds which represented the various stages in sex-reversal from female to male. It was found that the germ cells of the opposite sex proliferate from the peritoneal epithelium of the degenerating ovary as well as from the peritoneal epithelium of the vestigial right ovary. These cells form sex cords which in due time give rise to seminiferous tubules. The sperm cells from such tubules are known to be functional. The author concludes that "we have here a case of direct transition of ordinary somatic peritoneal cells into germ cells."

Similar observations have been made by Gatenby (1916), Firket (1920), and by Swingle (1921). In the light of our present knowledge, it may indeed be questioned if definitive germ cells ever are lineal descendants of primordial germ cells in higher animals.

It has been claimed that the peritoneal epithelium covering the ovary is "packed" with primordial germ cells which have reached the epithelium by migration and that those are the primordia of sex cord formation. Whatever might be the validity of this claim in case of birds the writer is not in position to say, but merely wishes to point out that the claim is inapplicable in case of *Xiphophorus helleri*. The primordial germ cells reach the body cavity en masse by passive displacement brought about by developmental processes of adjacent musculature and at no time do germ cells fuse with cells of peritoneal epithelium.

Causes of Sex-Reversal.

A case of complete sex-reversal in ring dove was described by Riddle (1923). The autopsy revealed that the viscera of the bird were seriously affected by tuberculosis which, together with other cases observed by Riddle, led him to the conclusion that tuberculosis is the immediate cause of sex-reversal. Based on the fact that the male bird has a higher metabolic rate than the female, Riddle assumes that the function of tuberculosis is to increase the metabolic rate of the diseased female bird. When once the metabolic rate has been increased, the diseased animal, according to Riddle, has nothing to prevent it from acquiring the anatomy and physiology of the opposite sex.

The data gathered from fishes undergoing sex-reversal do not seem to favor the claim that there is an increase of metabolic rate prior to, or during, transformation of sex. Indeed there are definite indications that the opposite is true. The transforming fishes become very inactive, probably take no food during early stages and are incapable of adapting themselves to changing environment even if the change be very slight.

Another case of complete sex-reversal in birds was describe by Crew (1923). This bird, a Buff Orpington hen, was also affected with tuberculosis of the viscera. Crew claims that sex-reversal is preceded by some disease such as tuberculosis, hemorrhage, tumor growth, etc. The disease affects the ovarian secretion and the general metabolism of the bird in such a way that the conditions favorable for the differentiation and growth of spermatic tissue are created.

It is definitely known that the fishes described in this paper were not diseased, certainly not by tuberculosis. Every transformed fish has been sacrificed to establish this very point. A number of diseased fishes have been observed, but none of these have become subject to sex-reversal. A case of complete sex-reversal in the domestic fowl is described by the writer (1926) in which no disease whatever is discovered. From the above fact the conclusion is warranted that disease is not a necessary precursor of sex-reversal.

It seems unquestionably true that the factor or factors which determine and control sex must also determine and control sex-reversal. It seems equally true that such factor or factors are of labile rather than fixed nature.

The chromosome theory has been considered as an adequate explanation of sex determination and control. It is assumed that sex is determined at fertilization at which the zygote receives the male or female constitution which it maintains for life.

In the light of data accumulated for such studies as hermaphrodites, intersexes and particularly from cases of complex sex-reversal, the chromosome theory seems to have reached the limit of flexibility. It is difficult to see how a definite chromosome composition which determines and controls sex can be completely overridden and still remain as a sex-controlling mechanism. The inadequacy of the chromosome theory finds expression in the often reiterated statement that "sex is not irrevocably decided by the sex-chromosome constitution." It seems logical to expect that there must be something that does decide sex and possibly the 'sex-chromosomes.'

Proof that maleness and femaleness depends upon the secretions of the testes and ovary has been supplied by a number of investigators. Perhaps the clearest cut data have come from castration and transplantation experiments, particularly from the work of Zawadowsky (1922). This author finds that after a total castration·of a young cockeral he loses his voice, sex instincts, and head-furnishings and thus assumes an asexual or generic appearance. However, if an ovary is successfully implanted into such castrated male, it will not only assume the appearance of the hen but the graft produces ovocytes as well. In case a young hen is castrated, she will acquire cock-feathers and spurs after the first molting. If however, the castration is incomplete, and the ovary has the chance to regenerate, the bird regains its female furnishings. Hens with total removal of ovary, develop testes as well as external furnishings of the male within four to five months. Castrated hens with implanted testes acquire male furnishings and if the transplantation is successful, the graft produces active spermatozoa.

From these and other experiments of similar nature which he has carried on with mammals, Zawadowsky concludes as follows: "Aus meinen Versuchen bin ich geneigt Hinweise darauf zu ersehen, dass hinter den Symbolen der 'Geschlechtsgene' F (female) und f (male) die geschlechts hormonen- Feminisin und Masculinisin zu suchen sind."

With the above statement the present writer is in full accord and wishes to add that with the replacement or substitution, if you please, of feminisin or the female hormone for the gene-anatomy represented by X and masculinisin or male hormone for the qualities of Y, the problems of sex are brought in accord with present knowledge of bio-chemistry and endocrinology. By so doing we not only gain an insight into the mechanics of the various types of hermaphrodite, of "intersex," and sex-reversal cases, which thus far have been a source of puzzle and speculation, but we also gain a better understanding of the science of genetics.

In conclusion, it may be said that there is no particular disease associated with sex-reversal. Any adverse condition be it extrinsic or intrinsic, acquired or inherited, which tends to reduce the capacity for hormone production beyond a certain limit may automatically become the immediate cause in sex-reversal in the female of *Xiphophorus helleri.*

VI. Summary.

1. Two cases of complete sex-reversal, from female to male, in adult *Xiphophorus helleri* have been described.
2. It is definitely known that both fish gave birth to normal young prior to sex-reversal.
3. It is also definitely known that both fish fertilized virgin females which gave birth to young with sex-ratio typical of the species.
4. The transformed fish is indistinguishable from a "normal" male except body form which is that of a female.
5. The entire ovary disintegrates except the epithelium of the

ovarian cavity. From it cells proliferate and form sex cords in sex-reversal cases.

6. The epithelium of the ovarian cavity is derived from the peritoneal epithelium and at no time is infiltrated with primordial germ cells.

7. The position, structure and physiology of the testis of the transformed fish is identical to that of the "normal" males. The oviduct becomes the spermduct.

8. No tubercular lesions or lesions of any other disease were found in the arrhenoid fishes.

9. Chromosomes are not considered as having the function of sex determination and control.

10. Sex is determined and controlled by the sex hormones derived from the ovary and testes.

11. Any agent or condition which tends to decrease the capacity for hormone secretion becomes an immediate factor in sex-reversal.

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SCROTAL REPLACEMENT OF EXPERIMENTAL CRYPTORCHID TESTES AND THE RECOVERY OF SPERMATOGENETIC FUNCTION (GUINEA PIG).[1]

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The experimental work of Griffiths on the dog in 1893 proved that the testis of a normal breeding mammal, replaced in the abdominal cavity by operation, was converted within a few months into a degenerate testis devoid of an active germinal epithelium; the structure of such a testis was very similar to that found in testes congenitally retained in the abdomen. This structural condition, differing from the general opinion held until recently, is not the result of a faulty embryology but the result of an abnormal environment; the structural counterpart of the congenital abdominal testis can be duplicated in a very short time by moving the adult normal testis from the scrotum to the abdomen. Griffiths appreciated the fact that the assumption of this abnormal structural character was due to a change of environment, but he was entirely ignorant of the nature of the environmental difference, or the condition that contributed to the typical reaction.

In a restudy of this problem of experimental cryptorchidism (Moore, '24*a*) and many other aspects of the biology of the testis, we have obtained considerable information concerning the environmental influences and reactions within the mammalian testicle when this environment is modified. Since the literature on the biology of the testis and scrotum has recently been reviewed and discussed (Moore, '26*a*) only a few points of importance to the present study need here be mentioned (see also Moore, '26*b*, *c*, *d*). We have determined that a functional testis removed from the scrotum to the abdomen does not require months to show the destructive influences of the abdominal position, but decided disorganization of the seminiferous tubules occurs within six days (guinea pig); the destructive effects are so pronounced and progressive that the testis is almost entirely cleared of its germinal epithelium within twenty to thirty days. As degeneration becomes more pronounced the testis as a whole diminishes markedly in size; the individual diameter of the seminiferous tubules is less; and usually the interstitial cells become decidedly more prominent. The testis does not again become active in spermatogenesis if it remains in the abdomen; two years after elevation it is but a small fraction of its former size, and entirely inactive in germ cell production. We have shown by many different lines of evidence that a higher temperature in the abdomen is the fundamental cause of its conversion into a degenerate testis and hence the scrotum has been recognized as a local thermoregulator which functions to produce a lower environmental temperature about the testis; it follows that such a regulation of temperature is necessary for the normal activity of the organ (Moore, '24*b*, Moore and Quick, '24).

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It was pointed out (Moore, '24*a*) that replacement of a testis in the scrotum, shortly after it had been caused to degenerate, was followed by restoration of its gametogenetic function; figures representing the grade of degeneration at the end of twenty-four days abdominal retention and the recovery within a few months after scrotal replacement reveal the reëstablishment of spermatozoön formation. This emphasized the fundamental importance of the scrotal regulatory function on spermatogenetic activity.

The question has been raised many times since this publication whether a testis congenitally retained in the abdomen in man could later assume its normal function if it were replaced in the scrotum by an operation. And in attempting an answer to this important problem, further study has led to observations that form the basis of this report.

MATERIAL AND METHOD.

The most usual cryptorchid condition encountered in nature (in man or other mammals) is a unilateral abdominal retention with the opposite testis normal; obviously the testis at fault has never descended from the abdomen. In the guinea pig descent of the testis occurs before or immediately after birth, and in order to reproduce as nearly as possible congenitial abdominal retention, the animal must be operated very soon after birth. Guinea pig testes from five days to twenty days after birth (sometimes later) show but little tendency to differentiate into the elements of the germ cell line and usually spermatozoa are not found in the seminiferous tubules until from forty to sixty days of age. Operation can, therefore, be delayed until the animal has become quite self-supporting and the testis confined to the abdomen considerably in advance of tubular differentiation; with such procedures we are able, therefore, to reproduce essentially the conditions of congenitial retention.

The question of single or bilateral elevation of the young testis was definitely solved by our earlier experiments. It was pointed out that an abdominally confined, prepubertal testis reacted differently if its partner was normal or if it were either removed or likewise confined in the abdomen. When one testis is carrying on its normal gametogenetic activity a prepubertal testis in the abdomen remains essentially in an embryonic resting condition for months and even years; the seminiferous tubules retain essentially the same diameter, the cells do not perceptibly change their embryonic character, the interstitial spaces remain in fundamentally the same condition as at the time of elevation and the testis as a whole does not increase in size. If, however, both prepubertal testes of an animal are elevated to the abdomen, or if one is so elevated and the opposite testis removed, then the reactions are of a different character. The abdominal testis does usually increase in size, and the tubules in their diameter, but such an increase is essentially due to an edematous condition of the testis as a whole; the cells of the seminiferous tubules instead of retaining the embryonic condition become differentiated into cells believed to be Sertoli cells; the interstitial spaces are often much larger than the embryonic type and usually contain numerous and conspicuous cells of Leydig. This type of testis is not an embryonic type, but one that has undergone considerable modification. It is apparently very definitely indicated that the experimental animal should have but one testis displaced into the abdomen while the other develops normally, and this report is restricted to material of this operative history.

The operative procedures, with perhaps minor variations, have consisted in opening the abdomen in a mid-ventral incision, retracting one testis into the abdomen, loosening its connections with the scrotal pouch, and fastening the testis high up in the abdomen by a suture passed through the epididymis and fastened to the body wall; this prevents possible return to the scrotal pouch, inasmuch as the inguinal canal was left open for future operative return of the testis to the scrotum. The opposite testis remained normal throughout the experiment or until the second, or replacement, operation at a later date. Four to five months after testis elevation the abdomen was opened a second time, the ligatures and adhesions cut and the testis replaced in the scrotal pouch in its usual position; a suture passing from the epididymis through the scrotal wall to the outside assisted in holding the organ in its desired location. Replacement of the testis without scrotal injury is not always successful. The absence of the growing testis from the scrotum results in a smaller pouch and coupled with this factor is the great increase in the fat body attached to the testis; often the testis to be replaced is but a fraction the size of its appended fat body. Attempts have been made to cut away the major portion of the fat body, without undue injury to the vascular plexus located within it, but the formation of adhesions of the entire mass replaced in the scrotal pouch have many times prevented a proper realization of the powers of the gland for recovery; adhesions often interfere decidedly with normal testicular function.

In most cases the opposite normal testis was removed at the replacement operation and on some occasions the degenerate testis was carried to the opposite side and replaced in the position of the normal testis in the larger scrotal pouch; the vas deferens often prevents the testis from reaching the bottom of the opposite scrotum and in some cases it has been purposely cut away to avoid such interference. It is now known that the testis does not have to be connected with its vas deferens to produce spermatozoa. The testicular blood supply must of course remain undisturbed.

From three to five months after replacing the degenerate abdominal testis in the scrotum, the animals were killed and the testes so preserved and sectioned in paraffine as to make available entire cross sections of the organ from different regions. In some cases tests for motile sperm in the epididymis were made immediately after death. In every case autopsy revealed the replaced testis in a somewhat unfavorable condition for normal activity in as much as adhesions with the tunica vaginalis had formed; many times the testis with its fat body was firmly attached by adhesions to the bottom of the scrotum. Under such conditions the growth of the fat body undoubtedly brings excess pressure to bear upon the organ and in many cases the entire replaced testis was practically surrounded by fat. All such abnormal conditions minimize the chance of the testis being able to acquire its complete gametogenetic function. In a few cases adhesions were so formed that the position of the replaced testis was the equivalent of an inguinal canal retention or even lower abdominal retention; under all conditions, however, a portion of the testis came within the upper limits of the scrotum and was subjected to some extent to its influences.

Observations.

The observations here reported are based upon the most recent series of operations, involving a group of ten guinea pigs, in each of which one testis was removed from the scrotum to the abdomen at intervals from the 17th to the 42d day after birth involving no interference with the opposite testis (see Table I.). In nine animals the unilateral cryptorchid testis was replaced in the scrotum four or five months later; the tenth unilateral testis was removed at 4½ months abdominal retention (animal No. 5) and prepared for histological study to serve as a control for the type of testis replaced in the scrotum of the other nine animals. In seven animals the opposite normal testis was removed during the operation for replacement of the cryptorchid testis and in the other two (Nos. 3 and 7) the normal testis remained intact throughout the experiment. The animals were

TABLE I.

No.	Animal Series.	Testis Elevation, Days after Birth.	Testis in Abdomen.	Testis in Scrotum.	Spermatozoa at Autopsy.	Remarks.
1	140(199)	17	4 months	$4\frac{1}{2}$ months	present	Normal testis to 2d oper.
2	141(200)	17	4 "	$4\frac{1}{2}$ "	present	" " " " "
3	142(201)	21	5 "	3 "	present	Normal testis throughout.
4	143(202)	21	5 "	3 "	present	Normal testis to 2d oper.
5	144*A* (211)	30	$4\frac{1}{2}$ "	—	—	Killed at $4\frac{1}{2}$ months (Control) normal testis present.
6	144(208)	30	4 "	3 "	absent	Normal testis to 2d oper.
7	145(213)	30	$4\frac{1}{2}$ "	5 "	present	Normal testis throughout.
8	146(209)	30	4 "	6 "	present	Normal testis to 2d oper.
9	147(197)	42	$4\frac{1}{2}$ "	10 "	present	" " " " "
10	148(198)	42	$4\frac{1}{2}$ "	10 "	present	" " " " "

sacrificed at intervals from three to ten months after scrotal replacement of the abdominal testis.

In order to give a better understanding of the conditions of these testes before scrotal return, I reproduce here Fig. 1 (127*A*)

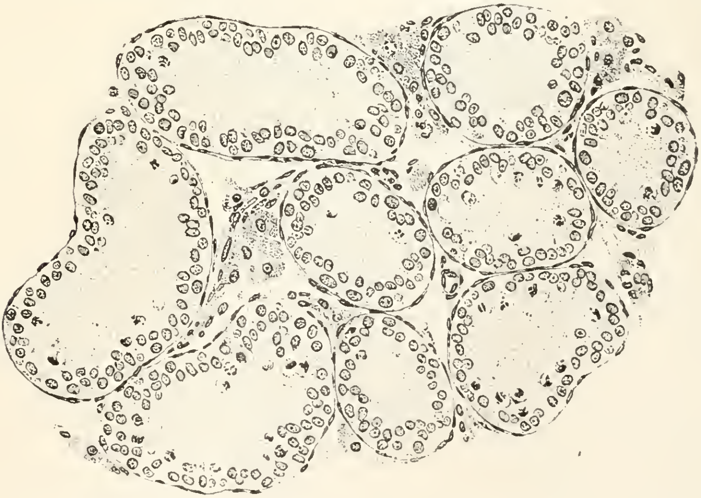

FIG. 1.[1] Seminiferous tubules of normal guinea pig testis seventeen days after birth (127*A*–218). Many mitotic figures but germinal epithelium shows little advance over embryonic condition.

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[1] All drawings made by Kenji Toda.

to show the structural condition of the testis shortly after birth. It can be seen that this 17-day normal guinea pig testis consists of seminiferous tubules that show little or no tendency to produce an active germinal epithelium. The testis is essentially of the embryonic type and differentiation into the different elements of the germinal line does not occur until considerably later. When such a testis as this is confined to the abdomen it tends to remain in about the same stage of differentiation for several months and in fact after two years abdominal retention quite a similar picture is presented, provided the opposite testis is a normal one. If, however, both testes are confined to the abdomen, this characteristic embryonic type is not retained.

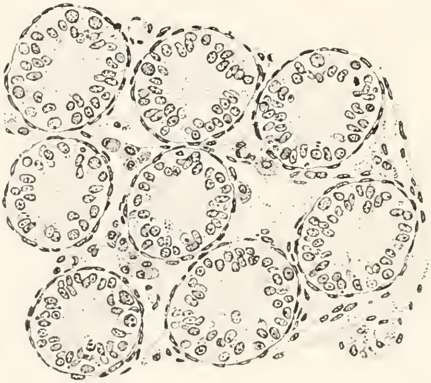

FIG. 2. Seminiferous tubules from a guinea pig testis (144*A*–211) elevated to the abdomen thirty days after birth (unilateral cryptorchid with opposite testis in scrotum) and removed $4\frac{1}{2}$ months later. (Same magnification as Fig. 1.)

Fig. 2 (144*A*–211) is a reproduction of a few seminiferous tubules from the unilateral cryptorchid testis removed from animal No. 5 (see Table I.). It was elevated to the abdomen on the 30th day after birth and remained for $4\frac{1}{2}$ months (opposite testis normal). Since this is approximately the average period of confinement in the abdomen for all cases reported here, it will serve to acquaint us with the grade of organization of all such testes at the time of their replacement in the scrotum. One can appreciate from Fig. 2 that although this testis was removed from the animal $5\frac{1}{2}$ months after birth, its structural development has remained essentially that of the normal testis of an animal seventeen days after birth; in other words the testis has shown practically no change from its condition at the time of elevation to the abdomen; it is prespermatic and has never produced a differentiated germinal epithelium (compare with Fig. 1) nor would it ever build such an epithelium were it allowed to remain in the abdomen. Returned to the scrotum, however, such a testis becomes active, builds a normal germinal epithelium, and produces motile spermatozoa.

In the nine animals (Table I.) in which the abdominally confined testis was replaced in the scrotum all but one (No. 6) subsequently produced spermatozoa. In two of the animals the testis was recovered after a period of but ninety days subsequent to replacement in the scrotum; two cases remained for 4½ months, one for 5 months, one for six, and two for ten months. Development under the scrotal influences has, therefore, produced a testis differentiating spermatozoa within ninety days after its return from the abdomen.

The success in development varies with the success in scrotal replacement. In every replaced testis adhesions of variable extent had formed. This not only serves to modify even normal testis activity, but it has resulted in a variety of scrotal relationships. Some of the testes were firmly bound down to the bottom, or against the side of the scrotal pouch, and surrounded by the fat body normally attached to the anterior end of the testis; some were held firmly in the upper portion of the scrotum or in the inguinal canal; other testes were found to have been held firmly against the upper entrance to the scrotal pouch in a position more abdominal than scrotal, but in all at least one side of the testis projected sufficiently low to be palpable in the upper scrotum. Roughly paralleling the success of replacement in the scrotum is the success of the testis in developing normal spermatogenesis.

Let us take for detail consideration, animal No. 7 (Table I.). The right testis was removed from the scrotum into the abdomen on the 30th day after birth and allowed to remain high up in the abdomen for 4½ months; the left testis was undisturbed. Since the operation periods were the same for this animal as that from which Fig. 2 was made, we can appreciate its structural condition at the time of scrotal replacement. Having remained in the abdomen for $4\frac{1}{2}$ months the testis from animal No. 7 was returned to the scrotum and allowed to remain therein for a period of five months. When the animal was killed this testis was firmly adherent to the bottom of the scrotum and more or less surrounded by its fat body. It cannot, therefore, be considered to have a normal environment on account of the constricting adhesions and the encapsulating fat. It was many times larger than when replaced in the scrotum and compared rather favorably in size with the normal testis opposite. From entire cross sections at three different levels one can see that the majority of the seminiferous tubules are in active spermatogenesis and a large percentage of these contain spermatozoa. Fig. 3 is a reproduction of a few normal seminiferous tubules,

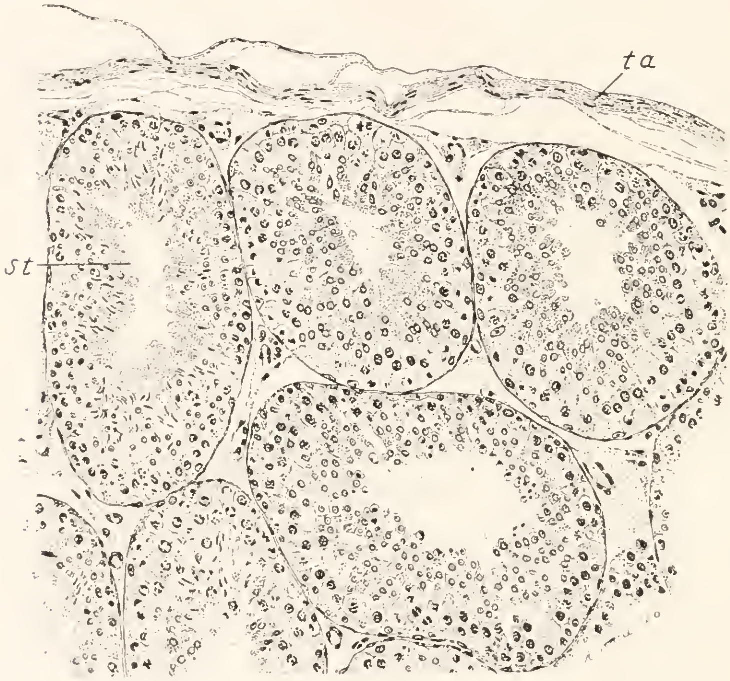

FIG. 3. Seminiferous tubules from guinea pig testis (145–213) elevated to abdomen thirty days after birth, remaining in abdomen $4\frac{1}{2}$ months, replaced in scrotum for five months. Complete recovery in this area. *st*, seminiferous tubule with spermatozoa; *ta*, tunica albuginea.

some of which contain spermatozoa, and this figure should be carefully compared with Fig. 2 representing the structure of this testis at the time of scrotal replacement.

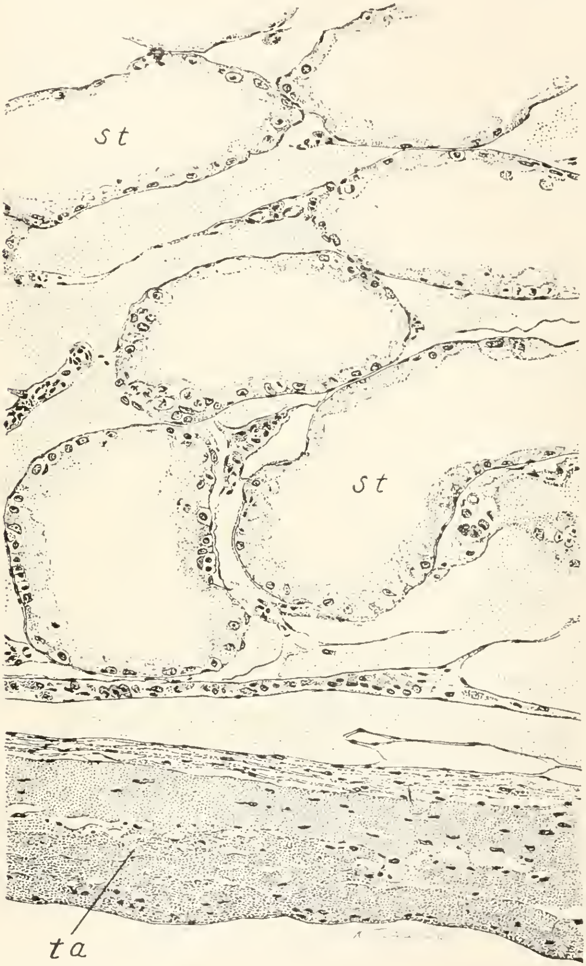

FIG. 4. Seminiferous tubules from same testis as Fig. 3 but from the side where adhesions were present. (Figs. 3. 4. 5 same magnification.) *st*, degenerate seminiferous tubules; *ta*, thickened tunica albuginea (adhesions).

Despite the establishment of normal seminiferous tubules throughout this testis since its replacement in the scrotum, there are many tubules that have not been restored to normal conditions. Such tubules are more or less restricted to certain regions of the testis and may be entirely devoid of cells in mitosis or cells that appear to be members of the germinal line. Fig. 4 shows a small area from the same microscopic section as that from which Fig. 3 was taken, but from the opposite side of the organ which had formed adhesions with the surrounding tissues; this portion of the testis, therefore, was not located in a sufficiently favorable position for resumption of germ cell activity. Here the tubules have not, and probably never would have, developed an active germinal epithelium. One cannot always account for the localized regions of degenerate tubules but these are almost invariably found on the side of the testis where adhesions of the tunica albuginea to surrounding structures have been formed, or on the side where the fat body has closely surrounded the organ.

These degenerate areas within such a testicle do not interfere with the function of the normal areas. Spermatogenetic activity is continuous in the latter and spermatozoa are differentiated and transported to the epididymis; sections of the latter portion show the tubules filled with spermatozoa. Suspensions of these cells in 0.9 per cent. NaCl at the time of killing the animals revealed the spermatozoa to be as vigorously motile as those obtained from an entirely normal epididymis.

The difference between an abdominal testis and one replaced in the scrotum for a few months is indeed striking (compare Figs. 2 and 3). The entire development of the active germinal epithelium in the case cited, has followed replacement of the testis in the scrotum within five months. In animals 3 and 4 the replaced testis were found to contain quantities of spermatozoa three months after scrotal replacement despite the fact that the testis had been confined to the abdomen for a slightly longer period than the one considered in detail above. Development of the entire germinal line follows, therefore, within three months after scrotal replacement and appears as well established quantitatively as after a period of ten months in the scrotum (animals 9 and 10).

In the case of animal No. 7 the testis partner to the one replaced in the scrotum remained normal throughout the experiment but it is not necessary that it should be present for development of the replaced testis. This is well shown in the experiment inasmuch as in animals 1, 2, 4, 8, 9, and 10 the normal testis was removed at the time of the second operation, and each of these six animals produced spermatozoa in the testis after replacement in the scrotum.

Out of the nine cases of scrotal replacement only the testis in animal No. 6 failed to produce spermatozoa. This testis was successfully replaced in the scrotal position, but numerous constricting adhesions had been formed and the testis recovered at the termination of its three months in the scrotum was considerably smaller than others residing in the scrotum for a similar period of time after replacement. Sections of this testis showed an entire lack of dividing cells in the seminiferous tubules; all cells present appear of the Sertoli type. It appears that all cells of the germinal line had been removed from the tubules and it is doubtful if this testis would have shown recovery effects if it had been allowed to remain in the animal for a considerably longer period.

The structure of the tubular portion of the testis in this case is typical of those cryptorchid testes that have not been opposed by a normal testis. Fig. 5 shows, in comparison with Fig. 2, that the tubules have undergone a change from the embryonic type to a type where apparently only Sertoli cells are present. The tubules are larger in diameter and lack undifferentiated cells, and the intertubular spaces are decidedly different from those of the undeveloped testis; interstitial cells of Leydig are prominent. Just why this testis should have failed to develop normal seminiferous tubules as did the other eight of the experiment similarly operated is not known unless it can be attributed to the constricting adhesions that developed about it after scrotal replacement.

The importance of the scrotum in controlling conditions that are essential for testicular activity can be shown by reference to conditions in which the testis was incompletely restored to the open scrotal pouch. In animal No. 1 scrotal replacement was incomplete inasmuch as the testis had developed adhesions and was held in a position essentially across the entrance into the upper portion of the scrotum; in reality it was more nearly abdominal than scrotal. On recovery, however, the testis was very evidently much larger than at the time of the replacement operation. Histological sections show that the majority of the seminiferous tubules were in active spermatogenesis, but very few of the tubules had developed to the stage of spermatozoön formation. Many of the tubules, though possessed of an active germinal epithelium, contained degenerating cells, multinuclear protoplasmic masses, and many vacuoles in the central portion of the epithelium. In other words, the testis was quite similar to testis grafts, heat applied testes, and subcutaneous testes in which spermatogenesis has continued but was usually never complete; the testis is not in an optimum environment for normal tubular activity. The few spermatozoön bearing tubules, however, show that the testis was in a position just on the effective borderline with a few tubules capable of completing spermatozoön formation.

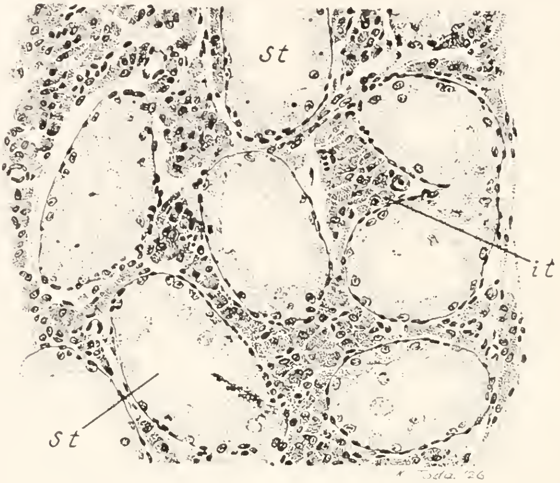

FIG. 5. Seminiferous tubules from guinea pig testis (144–208) elevated to abdomen thirty days after birth, remaining in abdomen four months, replaced in scrotum for three months (no recovery of function). *it*, interstitial tissue; *st*, degenerate tubules.

DISCUSSION.

A consideration of the observations here presented brings forcibly to our attention again the importance of the scrotum to the normal activity of the testes in those mammals for which a definite scrotum has been developed. It was pointed out earlier that testes caused to degenerate, after having once become active, by placing them in the abdomen for short periods of time would in some cases recover their normal function when replaced in the scrotum (Moore, '24*a*). Such a set of conditions, however, are obviously different from abdominal confinement before differentiation of the embryonic cells had taken place or from congenitally retained testes found in nature. And in order to gain an insight into the relative powers of undescended testes to acquire normal function after replacement in the scrotum, this series of experiments has been made; it may give us a relative answer to the possibilities of replacement of unilateral cryptorchid testes and recovery of function in man, but it is obvious that the answer is not absolute.

The conclusions are evident that a guinea pig testicle retained in the abdomen from shortly after birth until long after the establishment of sexual maturity, a condition essentially the same as a congenital retention of a testicle in man and a condition in which histologically similar testes are produced, can develop into a functional testis within ninety days after its replacement in the scrotum. Delay in the development of a testicle to its normal condition by abdominal confinement does not preclude its later development when it is brought into its proper environment. In regard to the application of such a conclusion from the guinea pig to essentially similar conditions found in nature, one must be very hesitant indeed in making more of the comparison than is justified. But if we may make so bold as to attempt a correlation between the findings here and their possible application to man, it would appear to be well indicated that we could expect scrotal replacement of a congenitally retained testis of a man long after the onset of sexual maturity, to produce a testis with normal spermatogenetic function within a relatively short period of time. In terms of years, it is more unsafe to predict the outcome. The guinea pig usually has produced spermatozoa in its seminiferous tubules within sixty days after birth. By these operations we have prevented this and held the testis in an undifferentiated condition for a period longer than four months, yet have had recovery of function on scrotal replacement; the implications from testes confined longer in the abdomen are that similar results could have been obtained from testes confined in the abdomen for over a year. Since three years may fairly well represent the average duration of sexual function in the guinea pig, one who is interested may carry the implications to relative periods of retention in the human individual and perhaps gain some insight to possibilities existing in such conditions. From the fact that recovery in the guinea pig may follow many months after the onset of sexual maturity, we might expect that in the human individual of twenty-five to thirty years, and from implications one considerably older, a scrotal replacement might result in the production of a normal testicle. It is obvious that these implications, though basically sound, require exact study before they can be more than implications. From the nature of the case it is obvious that since relatively few operations of this character are now attempted on the human individual, and since it is not frequent that such a replaced testicle will come to histological study, there is but little exact data in the literature of any significance in making such comparisons. Bevan ('18), among other surgeons, has given considerable study to the problems on the human individual, but it is well recognized that with one normal testis besides a scrotally replaced one, about the only criterion of effectiveness in its replacement would have to come from size and firmness determined by palpation or perhaps from hypodermic syringe samples.

The nature of the influence on a prepubertal abdominally retained testis by the opposite normal testis is not clear; the spermatogenetic activity, however, does exert some influence on the character of the undescended testis. Lipschutz ('25) has recently shown that the expression of the ovarian influence in a male animal by an ovarian transplant is likewise conditioned by spermatogenetic activity; a normal testis prevents the expression of the hormone influences of a transplanted ovary whereas a cryptorchid testis does not prevent it. The effect of the influence on a unilaterial abdominal testis is expressed by an almost entire suppression of any developmental capacities. In contrast to this the cryptorchid testis uninfluenced by spermatogenesis in another testis undergoes decided modification. In earlier reports (Moore, '24*a*) it was noted that replacement of abdominal testes that had undergone this change was not followed by normal gametogenetic function. It is believed that the degeneration of such testes had proceeded to the extent that all germinal cells were removed from the tubules.

The radical difference between the four or five month cryptorchid testis here described and the condition following within 90 days after replacement in the scrotum, serves as a striking demonstration of the difference between the capabilities of a given testis retained in the abdomen and one replaced in the scrotum. Having remained embryonic in character and relatively quiescent for months, it rapidly becomes engaged on a constructive program and fulfills the primary function of the organ before the end of three months. Thus the scrotal influence is necessary for the expression of the potentialities of the organ and the reader is referred to previous articles for the many lines of evidence that have led to the conception that the effective principle involved is a heat regulatory function on the part of the scrotum.

Summary and Conclusion.

1. Guinea pig testes elevated from the scrotum to the abdomen shortly after birth (prespermatogenic) will, if opposed by a normal testis, retain an undifferentiated character practically throughout the life of the animal. If unaccompanied by a normal testis, they undergo changes leading to the enlargement of seminiferous tubules lined with cells of Sertoli, relative increase of interstitial cells, and a general condition unlike that of the embryonic type.

2. Replacement in the scrotum of the testis retained in the abdomen for five months was followed by normal activity and differentiation of spermatozoa in eight out of nine cases. Presumably normal development of abdominal testes confined in the abdomen for much longer periods would follow scrotal replacement.

3. Spermatozoa are produced in the testis within ninety days after scrotal replacement.

4. Development of the replaced testis occurs in the presence or absence of an opposite normal testis.

5. By implication we are led to believe that congenitally retained abdominal testes in man have a good chance of developing into normal testes if they are replaced in the scrotum at a time considerably after the attainment of sexual maturity.

6. The function of the scrotum is beautifully shown by comparison of the testis retained in the abdomen for several months with a similar testis replaced in the scrotum for ninety days. Only in the scrotum are spermatozoa produced in the testes.

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THE FATE OF THE GERMINAL EPITHELIUM OF EXPERIMENTAL CRYPTORCHID TESTES OF GUINEA PIGS.[1]

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The experimental work of Moore and his students on the biology of the testis has yielded many interesting results and has pointed the way to some further analyses of the activities of this organ. Moore ('24*b*) has discussed at some length the reactions in a normal breeding guinea pig testis that has been removed from the scrotum and confined in the abdomen. The germinal epithelium rapidly becomes disorganized, undergoes degeneration, and the contents of the seminiferous tubules are almost completely removed within a very short time. Such a rapid degeneration is not followed by recovery of the generative portion as long as the testis remains in the abdomen; return to the scrotum, however, was followed by recovery to the extent that the testis again produced spermatozoa. This and many other determined facts emphasized the relation between a scrotal position and the germinal content of the organ and has led to the development of the idea that the scrotum is a local temperature regulator for the testes and that such a regulation is necessary for the production of spermatozoa (Moore, '24*c*; Moore and Quick, '24*a*).

Despite our general knowledge of the progressive degeneration of a testis placed within the abdomen we yet have much of importance to determine in the ultimate fate of the cells of the germinal line. Are the contents of the epididymis affected in the same manner and as rapidly as those of the seminiferous tubules? How long can spermatozoa be found in the genital passages of cryptorchid testes, and what is the relationship

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[1] This investigation has been aided by a grant from the committee on sex research of the National Research Council; grant administered by F. R. Lillie.

between morphology and motility of spermatozoa? These and other questions are discussed in the following pages of this paper.

It was at the suggestion of Professor Carl R. Moore that I attempted this phase of the sex problem, and I wish here to express my appreciation for this and for his kindly direction during the progress of the work.

OBSERVATIONS.

For the purpose of studying the progressive changes in the degenerating testis thirty-three guinea pigs have been used in which by operation one or both testes have been confined in the abdomen for relatively closely graded periods of from six to one hundred and thirteen days. The operation is simple and consists of opening the abdomen, freeing the testis attachment to the scrotum, and elevating the testis through the open inguinal canal into the abdomen; to secure permanency in this position the inguinal canals have been closed by sutures. The blood supply, nerve connections and ductus deferens are thus subjected to no interference.

A. SEMINIFEROUS TUBULE DEGENERATION.

Six-day Cryptorchid Testes.—Observed at the end of six days retention in the abdomen the testis of an adult breeding guinea pig shows but minor macroscopic changes; the organ may appear somewhat hyperæmic. Microscopical changes, however, are pronounced. The majority of the seminiferous tubules are abnormal, although the amount of alteration is variable. The diameters of the tubules are unchanged, and the intertubular spaces appear normal. In most cases the contents of the tubules are so shattered and disarranged as to bear little resemblance to the normal condition, while in a few the zones of spermatogonia, spermatocytes, spermatids and spermatozoa can be made out. On more careful examination individual cells within the tubules are seen to be swollen and granular, and in many cases more or less completely fragmented; numerous vacuoles appear among the cells constituting the germinal epithelium. From this it can be readily determined that some rapidly destructive process has involved all the tubules. The greater part of the lumen in most cases is filled with a mass of cells and debris, obviously in a state of parenchymatous degeneration. The intact cells are swollen and granular, and edges varying from perfectly smooth to a very irregular outline, and the nuclei likewise lose their identity; some of the latter appear eccentric, others fragmented, while in some of the degenerate masses no evidence of nuclei are to be seen. Of the degenerating cells in this central mass spermatids are most abundant, although many spermatogonia and spermatozoa may be seen. The latter, though least altered morphologically are most atypical in their position. Tufts of spermatozoa which normally border on or near the lumen are sometimes seen with their head almost touching the basement membrane of the tubule. Some of these masses are straight while others are adherent to the periphery of spheroidal masses of granular material. The other cellular elements have a tendency to coalesce and groups resembling typical giant cells of the myeloid type may be seen. The most common characteristic throughout all the tubules is the presence of large clear vacuoles in the germinal epithelium. These are most abundant near the periphery of the tubules, although they may be present in any portion of the cross section. Within some of these vacuoles fragments of more or less completely autolyzed cells are seen. There is little doubt that these vacuolar cavities represent areas of degeneration and liquefaction of epithelial cells.

We see therefore that a normal adult guinea pig testis removed from the scrotum to the abdominal cavity is decidedly degenerate at the end of six days. Six days after operation is the earliest stage studied, but it is apparent that the effects could have been noted at least one, or possibly two days earlier. In a given cross section of the entire testis, approximately 95 per cent. of the tubules are atypical, the remainder approximating the normal. In the most pronounced degeneration the germinal epithelium is so radically affected that it is in complete disorganization, and the lumen of the tubule may be all but obliterated by whole and fragmented cells from the epithelium.

Ten-day Cryptorchid Testes.—A testis retained in the abdomen for ten days is slightly smaller than the normal; this is clearly demonstrated where one testis is allowed to remain in the scrotum while its fellow is elevated to the abdominal cavity. In this case the cryptorchid testis is about four fifths the size of the normal.

The beginning degeneration noted in testes of six-day abdominal retention is now greatly advanced. The vacuoles which characterized the preceding stage have become more or less confluent, and in many tubules the desquamated epithelium is not so abundant, so that the lumen of the tubules is now larger and more open than in the previous stage. The lumen is not completely reëstablished in all cases; in many of them the central area is still occupied by wide vacuoles which have not as yet coalesced, while a few tubules are still cord-like in appearance. Cells showing advanced necrosis are present in the lumina of the seminiferous tubules and most of these are discrete, but some are agglutinated into masses which appear as a single large protoplasmic mass with multiple nuclei, the so-called giant cells. Of these degenerating elements within the lumen, many can be identified as spermatocytes and spermatids, but not as spermatozoa. Around the periphery, however, many spermatogonia and spermatocytes still remain, while an occasional spermatozoön may be seen not far from the basement membrane.

The blood vessels are somewhat more dilated in this testis than in the normal and an extravasation of serous exudate into the intertubular spaces, and a diapedesis of leucocytes from the capillaries may be seen.

A testis retained in the abdomen for ten days is, therefore, considerably more degenerate than one retained for six days, the chief difference being that in the ten-day retained testis there has been further degeneration of the cellular elements with a widening and often a confluence of the vacuoles.

Sixteen-day Cryptorchid Testes.—The organ appears about two thirds the size of an unoperated testis, and presents no superficial evidences of congestion. Microscopically the tubules show a decrease in diameter, as well as a diminution in the germinal epithelium content. The lumen in most cases is narrowed or even filled in completely by a thin reticulum of Sertoli cell cytoplasm. The number of Sertoli cells is apparently increased, due in all probability to the smaller caliber of the tubules, and a concentration of these cells, and they appear here as a simple epithelial lining for the tubules. With the exception of a few spermatogonia situated near the periphery of the tubules, intact germinal epithelium is practically absent. A few cells of degenerating germinal epithelium are seen free in the lumen; the rete tubules also contain some degenerating material that is being transported to the epididymis as will be pointed out below. The intertubular spaces show slight evidences of vascular stasis; the interstitial cells show no obvious change. The tunica vaginalis is somewhat thickened.

Thus by the end of the sixteenth day of abdominal retention, practically all of the former conspicuous germinal epithelium has been removed from seminiferous tubules, leaving chiefly a lining of Sertoli cells.

Twenty-day Cryptorchid Testes.—The testis confined to the abdomen for twenty days is smaller than the preceding organ, and measures slightly more than half the diameter of the unoperated testis in the same animal. The germinal epithelium is still more reduced here, so that only an occasional spermatogonium may be identified. In about half of the tubules the lumen is distinctly widened at the expense of the Sertoli cell cytoplasm, so that the lumen occupies from one half to two thirds the diameter of the tubule. The interstitial cells are slightly more conspicuous here and the intertubular spaces contain considerable serous exudate.

We see therefore that by the end of the twentieth day the process of evacuation of the germinal elements is complete, with the exception of a few spermatogonia, which retain their normal position between the bases of the Sertoli cells.

Abdominal Retention for Longer Periods.—Despite the fact that my interest has been primarily in the direction of the disposition of the degenerating epithelium, and that by the end of the twentieth day of abdominal retention practically all of this has been transported from the body of the testis, it has been found necessary to study the epididymis after longer retention. A word or two in reference to the seminiferous tubules of these testes, retained for from twenty-five to one hundred thirteen days, will not be out of order.

Moore ('24*b*) has already shown that a normal breeding guinea pig testis artificially retained in the abdomen loses practically all its germinal epithelium within twenty days and that it does not recover from this striking degeneration as long as it remains in the abdomen. My own observations confirm Moore's assertions that degeneration of the seminiferous tubules is marked after six days, that the degenerative process is progressive, and that by the twentieth day the tubules contain only the Sertoli syncytium within which are a few spermatogonia.

In my series of experiments testes retained from 25–113 days in the abdomen show no signs of seminiferous tubule recovery; indeed degeneration is more pronounced in that the testis becomes progressively smaller so that by the end of the second month the whole testis is but half the diameter of the opposite or normal organ. In these testes retained for two months or more in the abdominal cavity the diameter of the seminiferous tubules is greatly reduced and the interstitial cells occupy the greater part of the intertubular spaces; there is also more or less serous exudate between the tubules. The tunica albuginea is five or six times as thick as that of the normal testis.

It is apparent therefore that active degeneration of the germinal epithelium begins some time before the sixth day, is most rapid from the sixth to the tenth, during which time the greater part of the germinal epithelium is shed; succeeding changes have to do chiefly with the Sertoli cells which seem to be increased, the diameter of the tubules, and the relative abundance of interstitial cells. The rapidity of the transformation seems to be quite constant for all animals studied.

Vas Deferens Ligation of Abdominal Testes.—Seminiferous tubule degeneration, as pointed out above, is of a varied character; some cells are thrown out into the lumen, some apparently undergo solution in situ, the autolyzed products being absorbed directly into the blood stream. The question arose in reference to this degeneration, whether a part of the fragmenting cells and general debris in the lumen of the tubules were carried into the epididymis and eliminated from the body through the genital passages, or whether it was wholly or partially absorbed into the blood stream from the testis. To demonstrate just what elements are eliminated through the vas a number of animals were used in which both testes were elevated to the abdomen, and the vas deferens of one ligated in order to make certain the confinement of all testicular material to the organ in question. The degeneration of this vas-ligated, abdominally-retained testis was compared in its progressive stages with a similarly confined though unligated testis of the opposite side, and with testes with similar periods of retention in other animals. Macroscopically the two testes, one with ligated vas and the other with non-ligated vas when compared after any length of retention were entirely similar; one is unable to note difference in size or in vascularity. Microscopically also the two are similar. Degeneration begins as early in the testis with non-ligated vas as in the one with ligated vas, and the tubules of each are cleared of germinal epithelium in approximately the same time. So far as the seminiferous tubule degeneration is concerned, therefore, ligation of the vas deferens of an abdominally retained testis is without effect. The significance of this added ligation of the vas is demonstrated in the following section where the contents of the epididymis are considered. I had found that some of the degenerating cells, and some cells apparently not so degenerate were transported to the epididymis. Are any of these cells eliminated from the testis through the vas deferens, or do they go into solution in the epididymis and pass out entirely in the blood stream? Comparison of epididymal contents in testes of equal retention where one vas is open and one closed should give us an answer to this problem.

B. The Degenerative Process in Relation to the Contents of the Epididymis.

The epididymis forms a cap-like extension on the posterior pole of the testis, and is somewhat conical in contour, with the base adjacent to the body of the testis. Examined macroscopically in a normal pig it is seen to consist of tubules of varying size, largest at the apex and smallest at the base, and covered with a transparent membrane, the tunica vaginalis, through which the tubules appear as small while cords. The larger tubules at the apex constitute the ampullæ of the ductuli efferentes, which become smaller toward the base of the epididymis. These tubules in a sexually mature male are normally distended with spermatozoa which are suspended in a viscous matrix. On opening one of these normal tubules the contents exudes as a thin white paste-like substance which on microscopic examination reveals innumerable spermatozoa; these cells are usually immotile until a drop of physiological saline solution is added, when they are activated to rapid motility. Anderson ('22) in a series of experiments on the spermatozoa of various domesticated animals finds that sperm are normally immotile until they are activated by the accessory secretions of the reproductive system, but that the same results may be obtained by adding normal saline. Armstead ('25) working with guinea pigs finds sperm are made motile by addition of exudate from the seminal vesicles which action he attributes to the alkalinity of the exudate. I have observed slight motility in freshly expressed spermatozoa, even before the saline was added but this was a weak vibratile motion. When the saline is added the spermatozoa became very motile, and are seen to swim about in the medium in groups of from two to eight or ten, the heads being cupped together, not unlike the condition seen in blood where the erythrocytes have a tendency to form rouleaux. In this stacked condition the flagella point in the same direction, but there appears to be no rhythm in the motion of the flagella, some vibrating vigorously and some little or none at all; as a consequence the locomotion of these sperm groups is slow and cumbersome, particularly so in the larger groups.

In a cross section of the normal epididymis of the guinea pig the tubules are all seen to contain a greater or less number of spermatozoa with a marked tendency for agglutination as already described. The ductus epididymis contains a mass of sperm usually centrally located; the ductuli efferentes are more completely filled and the ampullæ of these tubules are thin walled sacs well distended with sperm. The ductus deferens, likewise contains a large number of sperm throughout its course.

Epididymis of Six Days Abdominal Retention.—Macroscopically the epididymis of a six-day abdominally retained testis shows slight differences in size and color from the normal. It is slightly smaller and its base has a somewhat glassy appearance, whereas the normal epididymis is milky white throughout. The contents as seen in cross section of the distal portion are very similar to those of the normal epididymis, and are distinguished by a few more round cells than are to be found in the normal. Toward the base of the epididymis the number of round epithelial cells is seen to increase, and along with these a considerable amount of degenerating debris all intimately mixed with varying amounts of spermatozoa. These germinal epithelial cells, in various stages of degeneration, have been carried over from the testis as a result of the liberation of these elements from the germinal epithelium through abdominal retention. Spermatozoa in the distal portion of the ductus epididymis and the vas deferens were similar to those of normal testes.

As it is important to know the effect of abdominal retention on the spermatozoa contained in the epididymis at the time of its removal from the scrotum, stained smears from the epididymis and vas deferens have been prepared in all cases after abdominal retention. Physiological saline has also been added to freshly expressed material from these localities in order to determine the duration of motility of spermatozoa. Mallory's triple stain has proven especially valuable here, as the head caps stain blue while the rest of the sperm body is stained red. Smears from the epididymis of a six-day abdominal testis show the sperm to be morphologically normal, and occurring in clusters as described above. On addition of saline the spermatozoa were seen to be as motile as the normal, moving about in the characteristic clusters, showing that as far as one can detect, the spermatozoa contained within the epididymis are in no way effected by the abdominal retention of six days.

Epididymis of Eight Days Retention.—A testis retained in the abdomen for eight days shows the base of the epididymis slightly glassy in appearance. In section the distal portion of the ductus epididymis was seen to contain an abundance of sperm, while in the proximal portion of this tubule degenerating elements predominate. For the most part the ductuli efferentes contain no cellular elements, being filled with a homogeneous liquid which stains faintly. In certain areas of the epididymis, and especially between the coils of the ductus epididymis are many polymorphonuclear leucocytes and indeed these are within the tubule in some areas. Ligation of the ductus deferens at the time of testis elevation produces no outstanding differences in the epididymal contents.

Sperm expressed from the ductus epididymis and ductus deferens as well as sperm observed in the prepared material are seen to be morphologically intact, but they have lost some of their tendency toward agglutination. When physiological saline was added to these spermatozoa, only a slight amount of motility was observed and this was a weak vibratile motion. No difference in the morphological or physiological characteristics in the sperm from the epididymis and vas could be discerned, nor was there any difference between the sperm from the ligated and non-ligated sides. It should be remembered, as pointed out above, that the seminiferous tubules of the testes are at this time at the height of degeneration with the lumina packed with degenerating remains, and that but few sperm could be found among these; transfer has continued from the seminiferous tubules since the sixth day, many of the degenerating cells being present in smears and sections of the epididymis. The sperm seen here are undoubtedly at least eight days old, and their viability is a point not to be overlooked.

Epididymis of Ten Days Retention.—The gross appearance of these epididymi is similar to that seen in the preceding stage. Where the vas is ligated the epididymis is somewhat larger and the basal portion shows a clear zone. At the end of ten days the ductus epididymis is fairly well distended with sperm, very little diluted with other constituents; this is true of both the ligated and unligated sides. In the proximal portion of these tubules fewer sperm are present with an abundance of spherical epithelial cells from the seminiferous tubules, and are characterized by their tendency to form spherical masses. These cells are identical with those described as giant cells in the degenerating seminiferous tubules. Some of the proximal tubules contain deep staining particules of irregular shape and dimensions immersed in a homogeneous coagulum. Sections show that in the proximal tubules in these epididymi the sperm have lost all tendency toward clumping, but in the distal portion this tendency is still present; however, smears made from this stage show no tendency toward clumping, and no sperm motility could be observed when physiological saline was added. In neither the epididymis nor the vas did the sperm show any obvious morphological change. It seems apparent therefore that as a criterion by which to judge of viability the tendency toward clumping is a better index than the morphology of the individual sperm.

The Epididymis of Sixteen Days Retention.—The epididymis retained for sixteen days in the abdominal cavity shows a glassy appearing zone three or four millimeters wide at the base. The entire organ is smaller than the normal, whether the vas is ligated or not, and where not ligated it is smaller and presents a broader clear zone at the base than in the ligated one. In sections the distal tubules are seen to contain spermatozoa which appear normal except for the clumping tendency. Further toward the base of the epididymis the number of spermatozoa becomes less, and at the extreme base there are few or none; instead there is to be seen a coagulum of fragmenting elements, degenerate beyond identification. Between these extremes some of the tubules contain a few spermatozoa mixed with a large number of swollen epithelial cells of varying size, among which are characteristic globular masses of these epithelial constituents. Polymorphonuclear leucocytes are common within and between the tubules in the basal portion of the epididymis.

In comparing the epididymis of the ligated and non-ligated vas, we find the latter somewhat smaller, the number of sperm less, and the amount of degenerating debris relatively more. This is easily explained since the ligature prevents the outward flow of sperm in the epididymis and vas, so that the degenerating constituents entering from the testis are able to occupy only the proximal tubules. The normal fellow of a cryptorchid epididymis is normal both as to appearance and contents and is apparently affected not at all by abdominal retention of the opposite testis.

The smears of the vas and epididymal contents from the testes of sixteen-day retention show sperm which are to all appearances normal morphologically, but which exhibit no pronounced tendency to clump, and are not activated to motility by the addition of physiological saline.

The Epididymis of Twenty-day Retention.—There is little difference between this and the preceding; the tubules proximal to the testis contain either degenerating debris, or sperm highly diluted with epithelial cells all of which are products recently evacuated from the testis. The distal tubules contain chiefly spermatozoa, and although most of these are normal, some show separate head caps and stain slightly fainter than freshly killed spermatozoa. It is a noteworthy fact that although the germinal epithelium of the seminiferous tubules is markedly disarranged and shows considerable degeneration at the end of six days abdominal retention, the mature spermatozoa present in the epididymis at the time the organ was elevated to the abdominal cavity show noticeable changes only after twenty days, a time when the seminiferous tubules are almost completely denuded of their germinal epithelia.

Twenty-five to One Hundred and Thirteen Days Retention.—With longer abdominal retention the vitreous area at the epididymis is increased, so that by the end of the second month the whole epididymis has lost its milky appearance. This difference in appearance is brought out by the fact that the liquefied and degenerating materials from the testis proper have mixed with the spermatozoa at the base and the latter have likewise slowly yielded to the degenerative process so that ultimately the entire organ contains only liquefied and fragmenting material which presents a vitreous appearance. That this material remains in the epididymis to be absorbed is shown by the fact that at the end of 113 days abdominal retention the tubules still contain a small amount of degenerating debris whether the vas is ligated or not. It should be mentioned here that the process of degeneration and absorption are much slower in the ductus deferens since sperm retained here proximal to the ligature are still identifiable after 113 days retention, whereas those retained in the efferent ducts are completely degenerated.

It is reasonable to suppose that with the addition of the degenerating products from the seminiferous tubules to the already distended epididymis that the latter would be distended beyond its normal size where the vas was ligated, or that an overflow would occur with the vas unligated. Observation, however, shows this to be true only in the early stages of degeneration, for in all cases of abdominal retention for longer than sixteen days the epididymis is actually smaller than the normal. This may be accounted for by the supposition that absorption rapidly removes the autolyzed portion of the degenerating material, and at the same time concentrates the contents already present in the tubules. On cutting one of these tubules and expressing some of the contents it is seen to be of much thicker consistency than that of the normal tubule.

Sections through epididymi at various levels and with varying periods of abdominal retention bear proof that the spermatozoa degenerate very slowly; this process, however, varies somewhat in the same organ, being slower in the thicker walled portion of the ductus epididymis and more rapid in the efferent ducts. In the vas sperm retained their integrity for 113 days, while in the ampullæ of the ductuli efferentes degeneration was apparent after 20 days. In the process of degeneration the spermatozoa first lose their head caps at which time they stain poorly, later the tail separates and the body undergoes granular degeneration.

Spermatozoa in Other Passages.—As a part of the routine examination of the urogenital passages of the animal at autopsy, the seminal vesicles and urinary bladder were examined by making fresh smears for the presence of spermatozoa. This was done by aspirating some of the contents of the urinary bladder with a hypodermic syringe and by opening the seminal vesicles at various levels and making fresh mounts of the contents. In no case were sperm found in the urinary bladder. In five cases sperm were recorded from a seminal vesicle; in two cases a single sperm was seen in each; in two cases the number was described as "few;" and in one case they were abundant. This observation was made before it was realized how very easy it is to carry over sperm from a severed vas on scissors or instruments. My observations, therefore, confirm the findings of Fisher ('23) and Armstead ('25) who demonstrated that the seminal vesicles in guinea pigs are not sperm reservoirs. In two cases, when the animals were killed by a blow on the head there appeared on the phallus a mass of material the size of a pea, which to the feel was very rubbery, and which upon examination was found to contain many motile spermatozoa, mixed with exudate from the seminal vesicles.

As a result of this series of observations it becomes apparent that the degenerating products of the seminiferous tubules due to abdominal retention are removed chiefly by way of the blood stream. My study leaves little doubt that a great deal of cytolysis goes on in the early stages of degeneration in the seminiferous tubules, and it is highly probable that these materials are removed from the testis by the circulation; but it is also apparent that a great deal of the degenerating material is transported to the epididymis. Since the tubules of the testis are cleared by the 20th day, and many times earlier, and since the epididymis, even with its vas normally open, contains much of its normal sperm content it is clear that the degenerating cells are not removed from the epididymis through the vas in any considerable amount. Indeed it is not apparent that the majority of the spermatozoa contained within the epididymis are so removed. My material leads me to believe that practically all the degenerating products from the seminiferous tubules are liquefied and absorbed in the blood stream, either from the testis proper, or the epididymis, and that a large part of the spermatozoa in the epididymis are likewise liquefied and absorbed.

C. The Vitality of Spermatozoa.

The question of the vitality of spermatozoa has been long debated, and extensive investigations have been made to determine just how long spermatozoa will live in the uterus; less attention, however, has been paid to the life of the sperm in the male genital passages. De Lee ('21, p. 21) states that human spermatozoa will live for 21 days in the human uterus. Nurnburger ('20) claims to have kept human spermatozoa alive in the laboratory for seven or eight days. Hoehne and Behne ('14) claim to have demonstrated that all sperm die in the human vagina in one and a quarter hours, and that in the uterus no live sperm may be found after three days. Lewis ('11) bred 20 sows to boars which were known to be fertile, after which the sows were slaughtered and the uteri examined. He states: "In only two cases were sperm found alive 24 hours after breeding." Anderson ('22) has pointed out that sperm are very sensitive to chemical and temperature changes. He was also able to find a few live sperm in the uterus of a mare seven and one fourth hours after breeding; and in hens sperm were found six to eight hours after breeding, but after fifteen hours none could be found. Wolf ('21) was able to keep rabbit sperm alive for nine days by the use of properly balanced physiological saline and isotonic glucose solution, and the required P_h concentration. Many more such references might be cited, but this will serve to show something of the diversity of the results obtained by different workers. Although there is doubtless a great variation in the viability of sperm from different species, doubtless some difference in results are attributable to the environment to which the sperm was subjected.

In this series of experiments particular attention was paid to the vitality of spermatozoa. When an animal was killed the vasa and epididymi were examined for sperm and where found were tried for notility by adding physiological saline to the contents. As stated above spermatozoa were found alive in the epididymis as long as eight days after both testes were elevated to the abdominal cavity, and closely associated with this there was a strong tendency toward dissociation of the clumps so characteristic of viable guinea pig sperm. To recheck on this very important point five pigs were used. Both testes were elevated to the abdomen, both vasa ligated, the left with a single ligature near the posterior pole, and the right in two places, one near the epididymis and the other one inch below, so that on the right side, the sperm which were in the lumen at the time of operation were not allowed to escape, and no more sperm could enter. When the animals were killed the contents of the epididymi, and of the vasa above, between and below the ligatures were examined and in each case the results were the same. The animals were killed on the 7, 9, 11, 13 and 14th days after operation. In only the first two cases, that is after seven and nine days were motile sperm found, and in these cases no difference in motility could be detected whether the sperm were taken from the region above, between or below the ligatures. In both these cases the motility was accompanied by the clumping phenomenon described in a preceding paragraph. Although there was motility in some of the sperm after nine days abdominal retention the per cent. which were motile was considerably less than in the case just cited. With the loss in motility the tendency to clump is lost, so that in sperm from a ten-day abdominally retained epididymis there is little or no clumping, although the sperm bodies may appear approximately normal for a month longer in the ductus epididymis and vas.

It is a surprising fact in view of the extremely rapid degeneration within the seminiferous tubule that spermatozoa morphologically normal are found in the epididymis for such long periods of time after all other elements have disappeared. Thus within twenty days the seminiferous tubules are approximately empty whereas the epididymis contains sperm apparently normal so far as morphology is concerned for thirty-seven days. It is also significant that the rapidity of degeneration of spermatozoa varies in different parts of the passages. Thus in the efferent ducts and in the proximal portion of the ductus epididymis degeneration is marked at twenty to thirty days, in the thicker walled distal portion of the ductus epididymis and vas sperm are considerably less altered after retention of 113 days. To account for this difference in rapidity of degeneration is beyond the scope of this paper, but it might be suggested that probably the cause is referable to the difference in vascularity of these parts.

Since in the vas, degeneration is least marked, and since this is the real reservoir for mature sperm, it would be reasonable to suppose that vitality would be retained as long or even longer than any place inside or outside the animal body, as here they are surrounded by what would be supposed to be the most suitable environment. As sperm are capable of showing motility for nine days in this location after ligature, and as these sperm were present an indeterminate length of time before operation, we can state with assurance that the maximum life of the guinea pig sperm in the male genital tract is more than nine days.

D. Vascular Changes in Relation to the Degenerative Process.

Having noticed that testes made cryptorchid for from six to sixteen days were somewhat hyperemic as evidenced by the dilated condition of the superficial veins and the presence of serous exudate in the intertubular spaces, it occurred to me that perhaps degeneration might be due to such a vascular change. Was degeneration being brought about through a reflex dilation of the vessels supplying the testis and if so, might not the same changes be produced through ligation of the internal spermatic vein by inducing passive congestion within the whole organ? Moore ('24*b*) also suggests that possibly some such relationship may occur. In an endeavor to demonstrate this relationship three pigs were operated; in two the internal spermatic veins were ligated and in the third the veins of the ductuli deferentes were ligated, care being taken not to occlude the ducts themselves. After seven days one of the pigs with internal spermatic vein ligature was killed. On the left side the ligature had not been made sufficiently tight to occlude the vessel, and the blood supply was found to be normal, but adhesions had followed handling the organ which was found within the abdominal cavity. In section this testis showed a very early stage in the degenerative process, earlier than that described for the six-day stage; and in this the contents were seriously deranged. Desquamation of the germinal epithelium had begun and spermatids were forming into giant cells in abundance. In some cases as high as forty nuclei could be seen together. On the right side, however, the testis was in the scrotum and the attached fat body was firmly adherent to the inguinal ring, thus preventing the return of the testis to the abdominal cavity. This testis was deeply cyanosed, the spermatic vein being completely occluded while the veins of the ductus deferens were greatly dilated. Microscopically this testis showed a vastly greater amount of hyperemia than any of the cryptorchid testes examined, yet there was nowhere the typical degeneration that was found in the abdominally-retained testes. There was no vacuolization nor emptying of the seminiferous tubules; instead, the tubular contents were considerably disarranged, swollen, and more or less coagulated in situ. The condition might well be compared to one of coagulation necrosis following infarction. The material stained very poorly with the stains which were being used. On the fourteenth day the other two pigs were killed, and in the case of the second, operated as above, the left internal spermatic vein was also successfully occluded and here also a condition similar to the one just described was found. Many of the seminiferous tubules contained spermatozoa atypically arranged as were the other contents, with the whole staining poorly.

In the case where the vein of the ductuli deferentes were ligated a new venous return was well established along the vas, and the testes were in full spermatogenic activity.

These observations indicate that although the hyperemic state may be closely coupled with the process of degeneration it is certainly not the causative factor. It would be admissable to suppose that the hyperemia is the result of the degenerative changes, wherein the autolyzed products are more rapidly taken up by the blood stream. It was noticed repeatedly that leucocytes were abundantly distributed throughout the intertubular spaces, and in many instances they were present in large numbers in the tubules of the epididymis. They are here doubtless concerned with the phagocytic function, and the degenerating epithelium which has been liberated from the seminiferous tubules are in effect foreign proteins which have perhaps stimulated this phagocytosis.

E. Discussion.

This work was undertaken to determine if possible, just how the germinal epithelium which is desquamated from the seminiferous tubules in artifically cryptorchid testes is disposed of, and incidentally to ascertain something concerning the relative resistance of the various cells of the germinal epithelium to the degenerative process.

As to the effect of abdominal retention upon the sexually mature male testis I have but to confirm the findings of Moore ably described in recent articles. The organ is effected in such a way as to cause the cessation of all spermatogenic activity by the end of six days; at twenty days abdominal retention the seminiferous tubules have been almost completely denuded of their germinal epithelium, and succeeding changes relate chiefly to the size of the testis, the diameter of the tubules, and the

Sertoli cell content. It has been abundantly demonstrated (Moore, '22, 24*c*; Moore and Oslund, '24; Fukui, '23) that heat of certain temperatures will produce in the testis a degeneration identical with that seen in abdominally retained testes, and that the degeneration is identical in nature and proportional within certain limits to the degree of heat, whether it be applied by hot water, hot air, parafine, electric lights, etc. And further it has been shown (Moore and Quick, '24*a*) that the temperature within the abdomen is definitely higher than intrascrotal temperature, all of which logically lead to the conception of the scrotum as a necessary thermo-regulator for the testis as developed by Moore ('24*c*).

Bouin and Ancel ('03) believe they have demonstrated tubular degeneration attributable to vas ligation. Others (Moore and Quick, '24*c*; Oslund, '24, '25) have been unable to produce degeneration by this method when the testes were carefully kept within the scrotum. Although in my work, testes, the vas of which were ligated, were elevated to the abdominal cavity where degeneration took place as a result of subjection to an elevated temperature, the testes so ligated were in all respect like those retained in the abdominal cavity the vas of which were not ligated. It is not proposed to offer this evidence in confirmation or derogation of the results obtained by any of these workers, but simply as a statement that in conjunction with abdominal retention ligation is without apparent effect.

It was pointed out in the text of this paper that although many of the epithelial cells in the seminiferous tubules are partially or almost completely autolyzed in situ, many others are thrown into the lumen of the tubule almost unchanged. These cells were followed through the straight tubules and rete to the proximal tubules of the epididymis where they are seen intermixed with spermatozoa already present previous to the elevation of the organ of the abdominal cavity. Obviously these invading cells, together with the spermatozoa already present must become absorbed in the epididymis, remain there, or be evacuated through the genital passages. Were the latter to take place the spermatozoa would be pushed on ahead probably mixed with some of the degenerating cells, followed by the newly acquired degenerating epithelium which should be found all along the passage. Were the whole to remain unchanged the epididymis would be ballooned up with both the old and newly acquired contents. The fact is the most of the spermatozoa present in the epididymis at the time of operation remain there, and are seen in various stages of degeneration within this organ. Likewise other germinal cells may be identified in the epididymis undergoing a more rapid degeneration than that seen in the case of the spermatozoa. We are left with little doubt that practically all the germinal epithelium of the testis and spermatozoa in the epididymis are absorbed directly from these organs into the blood stream after degeneration and liquefaction. As a further proof of this, if any is needed, spermatozoa which escape into the vas deferens which is markedly less vascular than the epididymis, require a much longer time for absorption than those retained in the ductuli efferentes.

And what of the effect of all this absorption on the organism? McCartney ('23) states that he was able to produce sterility in rats through injection of spermatozoa, and in a second article he states: "In the male rat vasoligation may be followed by the development of antibodies for semen in the blood." It is admitted that if the first is true the second would seem plausible. In my work ten pigs were operated in which one testis was elevated to the abdominal cavity, the opposite retaining its normal relation in the scrotum. The operated testes were allowed to remain for from six to sixty-four days in the abdominal cavity. Varying amounts of degeneration had taken place with the absorption of these products together with the spermatozoa present at the time of operation, and in not a single case was the spermatogenic activity in the opposite testis affected, nor was there any noticeable change in the testicular or epididymal contents from the normal.

Concerning the viability of spermatozoa so much has been said that any attempt to review the literature here would be futile. Be it said that reports vary from a few hours for the boar sperm in the sows uterus (Anderson, '22) to 21 days for the human sperm in the female uterus (DeLee, '21). In my observations I have found a fairly definite relation between viability and a tendency toward clumping. Spermatozoa were found alive in the ductus epididymis, and the vas for nine days after elevation of the entire testis to the abdominal cavity, and since it is reasonably certain that spermatogenic activity ceases on elevation of the organ, and since the spermatozoa pent up in the vas and epididymis were in these locations some time previous to operation, it follows that the length of life of a fully mature spermatozoön is something more than nine days. Just how long a spermatozoön remains in the seminiferous tubule after maturity, and what time is required for its passage into the vas is a question which must be answered before we can state anything more definite concerning the length of life of fully developed sperm.

As to the rôle played by the vascular changes in the degenerative process it must be admitted that insufficient work has been done in this direction to permit of any definite statements. However, from the meager data here presented it would appear that hæmostasis induced by ligation of the blood supply to the testis produces a coagulation necrosis of the germinal epithelium, not followed by a removal of the germinal epithelium at least within sixteen days. Hence we are lead to believe that the vascular changes seen in artifically cryptorchid testes is a case of active hyperemia, and that through this agency the degenerating elements are rapidly taken into the blood stream.

F. Summary and Conclusions.

1. When the testes of sexually mature male guinea pigs are elevated to and allowed to remain in the abdominal cavity, a progressive desquamation and degeneration of the germinal epithelium takes place; at six days abdominal retention degeneration is well marked, at ten days well advanced, and at twenty days practically all epithelium has been removed. This degeneration can be directly attributed to the increased temperature of the abdominal cavity over that of the scrotum.

2. Neither the speed nor the nature of the degenerative process is effected by ligation of the ductus deferens of the cryptorchid testis.

3. All of the degenerating elements from the seminiferous

tubules are absorbed in the testis proper, or are carried to the epididymis where they are absorbed along with the greater part of the spermatozoa which were present at the time the testis was elevated to the abdominal cavity.

4. Absorption of the germinal epithelium from a cryptorchid testis into the blood stream does not affect the integrity of the scrotally-retained normal testis.

5. Spermatozoa will remain alive in the germinal passages of the guinea pig for longer than nine days. Following their loss of motility they rapidly lose their tendency to clump, but degeneration of mature spermatozoa takes place slowly, and especially so in the ductus deferens. It has been determined that a given spermatozoön in the male genital passages though retaining its capacity for motility for nine days, may retain its morphological identity for over 100 days.

6. Venous stasis through ligature of the internal spermatic vein of testes allowed to remain in the scrotum does not produce the degeneration complex seen in testes retained in the abdominal cavity.

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BIOLOGICAL BULLETIN

OBSERVATIONS ON THE RELATIONSHIP BETWEEN A RED TORULA AND A MOLD PATHOGENIC FOR *DROSOPHILA MELANOGASTER.*

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In cultivating *D. melanogaster* at the Institute for Biological Research at Johns Hopkins University, a difficulty arose during the summer of 1925, which at times interfered with the growth of the flies. This difficulty was the appearance, in the flasks used for cultivation, of a growth that resembled the ordinary blue-green *Penicillium* but which appeared to form on the surface of agar, a thick, tough, basement membrane of a bright lemon-yellow color. This growth was apparently very obnoxious to the flies for they died in large numbers soon after being put into jars containing it. The phenomenon was brought to the attention of the author and the following studies made.

Microörganisms pathogenic for flies of various species have been described (1, 2). It was thought, therefore, that the present phenomenon might also be due to the presence of some pathogenic organism in the jars. With this idea in mind an attempt was made to isolate such an organism from one of the jars in which the flies had died very rapidly, and in which the yellow growth was particularly thick.

It has been observed by Pearl that flies might live in vessels containing growth of *Penicillium* of the ordinary varieties without any ill effects on the flies. Inasmuch as the mold in question was believed to be one of these ordinary types, it was thought that the obnoxious growth, if any were present, must be connected with the yellow pigmentation, since this had not been observed before and its appearance was connected with the death of the flies.

Material from the jar was therefore plated out on a medium similar to that used for the fly cultivation.[1] To obtain the inoculum, a piece of the tough, yellow membrane covering the surface of the agar in the fly bottle, under the growth of the mold, was cut out with a loop of stiff sterile wire. The under side of this piece of membrane was carefully scraped and the scrapings spread upon the medium in petri plates. Smears of the scrapings were made at this time, and stained with Gram's stain and with Loeffler's methylene blue. These preparations showed the presence of mold hyphæ, spores and yeast cells. No bacteria were seen. This is readily understood in view of the acidity of the medium.

Plates streaked as above were incubated in the refrigerator at about 6° C., also in the laboratory at a temperature of about 25° C., and other plates were held at 37° C. Growth occurred at all three temperatures, but most abundantly and rapidly on the plates held at about 25° C. On all plates there appeared nothing but the white commercial yeast referred to and the mold. There was no yellow growth on any of the plates, even after long standing at different temperatures and in the dark as well as in the light.

The attempt was regarded as a failure, since no organism appeared except those believed to be harmless to the flies. A second and third attempt yielded like results. No yellow organ-

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[1] This medium is prepared as follows:

Solution A.

Water Distilled	500 cc.
Sucrose	83.5 grams
$CaCl_2$	.25 "
$MgSO_4$	.50 "
$(NH_4)_2SO_4$	2.00 "
KH_2PO_4	1.00 "
Tartaric Acid	5.00 "
Rochelle Salt	8.40 "

Solution B.

Water Distilled	500 cc.
Agar	30 grams

Autoclave *A* and *B* separately. Mix aseptically in equal parts just before use. The solutions keep well separately. The reaction is about pH 3.2.

ism appeared and nothing even resembling the thick yellow growth in the original fly bottle could be seen.

Other fly bottles were obtained, and finally, from one of these, a growth was obtained which had the typical yellow color and the leathery texture. Upon replating this growth repeatedly, however, nothing could be obtained except the ordinary yeast and the mold.

After repeatedly smearing such material over plates and getting only the mold and ordinary yeast referred to, a final survey of all plates heretofore made was undertaken with a view to discarding most of them.

During this survey, however, there was noted on one of the older plates several tiny colonies having a distinct, coral-red color. One of these was fished to Saboraud's agar and held at room temperature for three days. During this time there appeared a soft, creamy, coral colored growth, which, upon microscopical examination, was found to be a yeast or torula. It was later shown that this red torula occurred as a contaminant in cakes of the commercial yeast referred to above. After three or four more days, the pink growths on the plates from which the pink colony had been fished,were observed to be gradually turning yellow in places, and wherever the yellow color appeared there was also a growth of the mold. The yellow growth had also become tough and leathery. Smears made from these colonies showed the presence of a mass of yeast cells matted and closely bound together by mold hyphæ. It appeared that the combined growth of the red torula and the white or unpigmented mold produced the bright lemon-yellow color, and the tough character of the colonies. It only remained to get pure cultures of the two organisms growing under controlled conditions to decide this point. This was easily done by inoculating one side of a plate with a pure culture of the mold and the other half with a pure culture of the red torulæ. The plate was held at about 22° C. for four days and good growth of both organisms occurred. The two growths remained separate for some days, but soon the spreading mold began to overgrow the area streaked with the torulæ. Wherever the mold encountered a colony of the torulæ, the colony became yellow, hard and tough.

One of these yellow colonies, smeared under a cover slip, showed that the mold had invaded the colony of torulæ and matted and bound the whole colony into a solid mass. The yeast cells seemed to suffer somewhat from the contact, since when one of these yellow colonies was stained by methylene blue most of the entangled cells appeared to be wholly or partly disintegrated. The destruction of the red torula cells by the mold probably explains why so much difficulty was originally encountered in isolating the red organism. The mold does not appear to have such an effect upon the commercial yeast, for good growth of this yeast was obtained even after prolonged contact with the mold.

Regarding the occurrence of a red torula in compressed yeast cakes it may be said that this is not a specially remarkable circumstance, and may have been observed before. The pink or red torulæ appear to be fairly widely distributed in nature. They have been known for a long time and seem to have been first described in 1850 when Fresinius (3) first described *Cryptococcus glutinis*. Pink torulæ or yeasts have been isolated from various beer and wine musts, milk, cheese, sea water, plant diseases and other sources. The finding of such an organism by Fisher and Brebeck (4) in the stomach of a case of gastric fermentation is of interest in view of the ability of the organism herein described to grow well at an acidity similar to that of the normal gastric contents. A pink yeast or torula causing spoilage in oysters has been described by Hunter (5). Similar organisms have been isolated by the author from oysters taken from Chesapeake Bay. The organism mentioned above resembles these varieties in many respects and probably belongs to the same family or genus, but also shows differences that indicate that it is not of the same species, the chief difference being that it is quite a strict aërobe.

A cultural study of the red torula yielded the following data:

Growth occurs at temperatures ranging from about 6° C. to about 37° C.

The optimum is in the neighborhood of 25° C.

Growth takes place on agar having a reaction ranging around Ph 7.4, and also as low as Ph 3.0. No very definite optimum seems to exist.

In a fluid synthetic medium the reaction at which good growth ceases was found to be about Ph 2.8, while that at which even sparse growth ceases was found to be about Ph 2.0.

Pigment formation seems to be slightly favored by the more acid medium.

The organism will grow on all of the ordinary media, with good pigment production.

Dextrose is fermented but slightly, even after 20 days growth. Acid is formed, but no alcohol could be detected by the method of distillation. No gas is formed. Lactose, saccarose, mannite and xylose are not fermented when used in extract bouillon.

Growth in a suitable synthetic medium of about Ph 3.2[1] occurs after five days as a dirty looking sediment. After standing for twenty-one days or more, however, heavy growth of coral pink sediment gradually appears, and, if left undisturbed, a thin pink scum forms, and a ring appears at the junction of the fluid surface and the vessel wall.

The torula is an aërobe. When an inoculated infusion agar slant was placed in a Brown (6) anaërobic jar, no growth occurred after ten days at room temperature. Upon removal of this slant from the jar, good growth occurred after forty-eight hours. A flask of the synthetic medium described above, which had been heated for some time in order to invert some of the sucrose, was also placed, after inoculation, in an anaërobic jar for one week. No growth occurred. Growth appeared quite promptly after removal of the flask from the jar.

Morphologically, the organism is ellipsoidal, the cells being from 5 to 7 μ long by about 3 to 5 μ wide. No spores have been observed. Typical budding occurs.

A study of the pigment showed it to belong to the class of acid-alkali indicators. The growth on an agar slant was treated with $n/1$ acid. No change in color took place. On treatment of the same growth with $n/1$ alkali however, the color turned to a brown-orange and the red color could be restored by further treatment with the acid. The change in color was not striking, but definite.

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[1] This medium is simply solution (*A*) of the agar described above, but containing 1,000 cc. of water instead of 500.

The growth on a Buchner flask was suspended in 50 cc. of water and left in the incubator for four days. None of the color was imparted to the water. It was found that no pigment could be extracted from the intact cells with ether, alcohol, chloroform, glycerine, or water. Nor was any to be obtained with these solvents after grinding the dried cells with sand.

Simultaneously with these cultural studies, attempts were being made to determine what had killed the flies in the original jars.

Pure cultures were made of the red torulæ and of the Penicillium-like mold in suitable flasks, and anæsthetized flies were introduced. The first three experiments are summarized in Table I. The red torula alone has since been used successfully to rear several vigorous batches of flies.

TABLE I.

FATE OF FLIES FED ON PURE CULTURES OF RED TORULÆ AND *Penicillium* RESPECTIVELY.

(Three experiments.)

Organism on which Flies Were Fed.	Exp. No.	No. of Flies Used.	Total Number of Flies *Dead* on Following Days:									
			1st	2d	3d	4th	5th	6th	7th	8th	9th	10th
Red Torulæ......	1	10	0	0	0	0	0	1	1	1	1	1
	2	10	0	0	0	0	0	0	0	0	0	0
	3	10	0	0	0	0	0	0	0	0	0	0
Penicillium.......	1	10	0	0	1	3	—[1]	8	10	10	10	10
	2	10	0	0	0	5	6	6	9	9	9	9
	3	10	0	—[1]	6	7	—[1]	10	10	10	10	10
Ordinary Yeast (Control)......	1	10	0	0	0	0	0	0	0	0	0	0
	2	10	0	0	0	0	0	0	0	0	0	0
	3	10	0	0	0	0	0	0	0	0	0	1

[1] No observation.

It has already been pointed out that the combined growth of torulæ and mold results in the formation of a hard tough mass. This led to the idea that the mold might kill the flies by growing among the yeast cells in the alimentary passages and thus block elimination and engorgement. It was found that a combination of the mold, and of the commercial yeast upon which the flies ordinarily engorge themselves with happy results, also produced a rather tough mass, but not nearly as hard and tough as the combination of the mold and the red torulæ. In cross sections of the gut of flies which had been allowed to feed first on torula and then on mold, and had then died, the gut appears somewhat distended with a matted mass of mold hyphæ and torula cells. It seems improbable that the fly could have taken in through the proboscis such large filaments, and it is believed that they must have germinated and grown in the gut after the ingestion of spores, or smaller portions of filament. Sections show that the mold hyphæ penetrate into the walls of the gut, either between the cells or into them.

This suggests that the matted mass of torula cells may be firmly bound to the intestinal wall by these adhesions of mold hyphæ. This would, it seems, add to the blocking effect. Further, the invasion of the intestinal wall might paralyze this organ so that elimination could not proceed normally on this account. A similar phenomenon has been described by Vincens (7) in the case of bees experimentally infected with a type of *Aspergillus*.

That this may not be the only means by which the mold kills the flies, however, was shown by placing bacteriologically sterile flies on mold cultures. In preparing the flies for this purpose a number of young pupæ were disloged from their moorings in one of the stock fly culture bottles and soaked for one minute in an aqueous solution of alcohol and mercuric chloride. They were then washed three times in sterile distilled water and placed, with a sterile loop, upon agar and kept three days at 37° C. and subsequently at about 22° C. After the adults emerged the vessels containing them and the agar were returned to the incubator for 48 hours and then removed. This was done to detect the presence of any bacteria which might have been inside the pupa cases and escaped the disinfectant. Most of the flies were found to be still contaminated, but some were not, and these were selected for use. The sterile flies were placed in a bottle containing a pure culture of the mold and in five days all were dead.

This rapid death pointed to either an effect of the mold, or starvation, or death due to drought, or all three.

In order to control the effects of starvation and drought on these flies, twenty-nine normal specimens were placed in a dry empty flask, ten in a flask containing nothing but a pledget of cotton wetted with distilled water, and thirty-four in a vessel containing sterile agar. The fate of these flies is shown in Table II.

TABLE II.

FATE OF FLIES UNDER CONDITIONS OF DROUGHT, STARVATION, ETC.

Conditions of Drought Starvation, Etc.	Number[1] of Flies at Start.	Total Number of Flies *Dead* on Following Days:									
		1st	2d	3d	4th	5th	6th	7th	8th	9th	10th
Dry, empty flask....	29	7	29	29	29	29	29	29	29	29	29
Wet pledget of cotton	10	0	6	10	10	10	10	10	10	10	10
Sterile agar........	34	1	1	3	—[2]	9	—[2]	17	17	17	17
Ordinary yeast culture (control).....	33	0	0	0	0	0	0	0	0	0	0

[1] Number varied because of activity of flies.

[2] No observation.

It appears that the flies die very rapidly as a result of drought and the absence of anything but water. The more prolonged survival of the flies on the sterile agar may be attributed either to an ability to metabolize this or its impurities or, more likely, to an automatic inoculation of the agar with the yeasts on their feet and in their feces.

It appears to be shown by this experiment that the sterile flies survive in cultures of mold beyond the period within which it would be expected that they would die of drought or starvation and it therefore seems logical to assume that they eat the mold, but that the mold finally grows within them and kills them.

Some of the dead flies were imbedded in paraffin within an hour after death and serial section made. Such sections show that the mold may actually invade the tissues of the fly.

It should be noted that error due to variation in longevity of the flies is probably nil, since:

1. The flies were all young (10–15 days old) progeny of standardized stock, originally furnished by Dr. Raymond Pearl, and deaths in these experiments always occurred well within the minimum of longevity.

2. The number of etherizations incident to these experiments never exceeded two in number nor more than 7 minutes in duration (8).

3. The conditions, under which the flies were reared and experimented upon, were practically ideal as to number in jars, ventilation, etc. (9, 10, 11).

Summary and Conclusions.

1. A red torula has been found as a contaminant of certain commercial yeast and a brief biological description given.

2. The combined growth of this torula and one of the group of Penicillium produces a bright lemon-yellow color.

3. Overgrowth of the torula by the mold transforms the creamy torula colony into a hard, tough mass, apparently by invading the colony and matting the cells together. The torula cells suffer by the contact.

4. The pigment of the torula has the properties of an acid-alkali indicator. Its sensitivity to these reagents is low.

5. The red torula may be used for raising batches of *D. melanogaster.*

6. A mold, probably closely related to *Penicillium glaucum* has been shown to have the power of killing *D. melanogaster.* The mechanism by which it injures the flies is shown to be possibly of two sorts. First, it appears to grow among the torula cells in the gut of the fly and by matting them together forms a more or less solid mass which may block both elimination and engorgement. By invasion of the intestinal wall, the mold hyphæ are believed to bind this mass firmly in the intestine. Second, it is believed that the mold actually invades the tissues of the fly.

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NUCLEAR REGENERATION IN *STYLONYCHIA MYTILUS.*

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In 1919 while working on the regeneration of lost parts in *Uronychia* which had been cut in various ways (cf. Young, *Jour. Exp. Zoöl.*, Vol. 36, No. 3, Oct. 1922) it was discovered that one species, *U. binucleata* had two micronuclei. When this form was cut in such a way that the two micronuclei were in separate halves each part regenerated completely and subsequently divided. At that time, however, no stained preparations were made to show the method of nuclear reorganization. In those species which had but a single micronucleus, *U. setigera* and *U. transfuga*, division never took place in the amicronucleate fragment although regeneration might do so. The question naturally arose, does the nuclear complex in those forms having two micronuclei regenerate at the same time that the cytoplasmic structures are being replaced and if so, how?

Uronychia binucleata has not appeared in any of my cultures recently but as *Stylonychia mytilus* has two micronuclei so placed that a cut in the median transverse plane passes between them, they have been selected for this study. A wild mass culture was used and individuals selected from it at different times as needed. Several of the normal individuals were stained in order to record their nuclear complex. In every case two macro- and two micronuclei were found. Animals which have just completed division can usually be distinguished by their smaller size. In the following experiments individuals were selected which were of average size, indicating that they had not divided for four or five hours. As the formation of precocious cirri can be readily seen and as animals do not show any nuclear changes until such cirri do form, it is clear that individuals were not used which had already begun the process of division. The cutting was done free hand with a small sharp scalpel. The fragments were carefully studied under the microscope and any in which the plane of cutting was such that either part of the nuclear complex might have been injured were discarded.

Seventeen animals were selected and cut in a median transverse plane, thus separating the two halves of the nuclear complex. The parts were kept under observation and the process of regeneration studied. In eight or ten hours each fragment had developed its missing structures. At the end of thirty-six hours most of the regenerated individuals had divided. Some of those which had already divided as well as some which had not yet started division were stained. In every case the nuclear complex was complete, that is, there were two macro- and two micronuclei.

Ten individuals were next cut in the same plane and in sixteen hours the pieces were stained. Regeneration was complete in both nuclear and cytoplasmic structures. No division had taken place. There was no indication of the method of nuclear reorganization.

Eight other individuals were stained about six hours after cutting. At this time the cytoplasmic regeneration was practically complete. The body form was normal and the number and arrangement of cirri correct, although some of the cirri were not full size. In each animal studied, the micronucleus was in the process of mitotic division and the macronucleus had begun to separate into two parts.

Eight individuals were stained three to five hours after cutting in the same plane as before. The cytoplasmic structures had begun to regenerate but the process was far from complete. The micronucleus showed various stages of division up to about a metaphase condition. The macronucleus in some cases had not begun to divide while in other cases it showed a slight constriction.

This study seems to indicate that in the race of *Stylonychia mytilus* used in this series of experiments the nuclear complex is constant and the whole complex is necessary for the normal life of the animal. Any cut which separates the two halves of the nuclear complex results in the regeneration of the normal complex by the amitotic division of the macronucleus and the mitotic division of the micronucleus. In every case studied, this process took place before the animal divided.

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THE CHEMICAL SENSITIVITY OF THE TARSI OF CERTAIN MUSCID FLIES.

(*Phormia regina* Meigen, *Phormia terræ-novæ* R.D.
and *Lucilia sericata* Meigen.)

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Introduction.

Experiments previously published by the author (Minnich, '21, '22*a*, '22*b*) have shown that the tarsi of certain butterflies possess sense organs which are exceedingly sensitive to contact chemical stimulation. These chemoreceptors are of such importance that a more general knowledge of their occurrence and function in insects is highly desirable. The data presented in this paper are the results of experiments on three common muscid flies, *Phormia regina* Meigen, *Phormia terræ-novæ*, R.D., and *Lucilia sericata* Meigen. For the identification of these flies I am indebted to Dr. J. M. Aldrich of the United States National Museum. The methods employed in these experiments are, in general, similar to those employed with butterflies, the results in both cases having been obtained by the use of proboscis extension as an indication of stimulation.

Material and Methods.

The experiments were carried out in the spring and summer months. In the first experiments, therefore, the flies were individuals which had hibernated, whereas in the later experiments they were individuals which had emerged the same season. All of the flies were captured, usually with a net, either from walls where they came to sun themselves in the early morning hours, or from waste cans to which they were attracted by the odor of decomposing organic matter.

The flies were transferred in bottles to the laboratory where each animal was etherized, its species and sex determined, and its appendages carefully examined to determine their state of perfection. If all six legs were not perfect, the animal was usually discarded. If the legs were perfect, however, the wings were cut off close to the body with fine scissors, and the animal was mounted on a holder. The holder consisted of a straight piece of telephone wire, 19 to 20 cm. in length, and flattened out to form a small plate at one end. To this plate was attached a rectangular block of beeswax deeply notched in the middle, thus leaving two projecting tongues. By careful manipulation, it was possible to heat the tongues one at a time with a hot needle, and while the wax was still melted to attach the thorax of the fly to one and the abdomen to the other, the entire operation being completed in about a minute. "Mounts" thus made are admirably adapted for experimental work and make possible the rapid handling of flies with the greatest ease (Fig. 1).

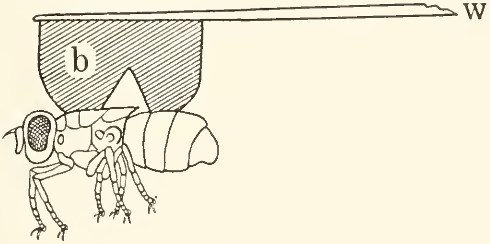

FIG. 1. Side view of a mounted fly. *b*, block of beeswax notched at center; *w*, wire flattened out at the end.

Immediately after mounting, the legs of each animal were carefully rinsed with distilled water to remove any foreign matter adhering to them. Recovery from the anæsthesia usually resulted in a few minutes, but a minimum period of two hours was allowed from the time of anæsthetization to the first trial.

As far as could be determined without special experimentation, mounting and experimentation failed, in the great majority of cases at least, either to injure the animal or to shorten its life to any appreciable extent. The animals were usually kept under observation for at least 24 hours after the conclusion of the experiment before being discarded. In one experiment, however, involving eleven individuals belonging to the two species of *Phormia*, the animals were kept on after the experiment until death, each animal being fed once daily with $1M$ saccharose solution. When the solution failed to effect a proboscis extension through tarsal stimulation, it was found that a response could always be obtained by touching the oral lobes of the proboscis tip to the solution. It was, therefore, possible to force every animal to bring the extended proboscis in contact with the solution even though it drank little or none of it. Two of the eleven flies died within twenty-four hours. The remaining nine, however, lived many days. Exactly, their longevities were 9, 10, 11, 28, 30, 33, 35, 44 and 54 days. Six of the animals, three males and three females, thus lived a month or more, the greatest longevity extending to almost two months. It is doubtful whether these six animals would have lived any longer, if as long, in a state of nature. (Compare Glaser '23.).

The use of proboscis extension as an index of tarsal stimulation entails a certain amount of care to avoid misinterpretation. Extensions of the proboscis are sometimes called forth by causes other than tarsal stimulation, e.g., cleaning operations, distance chemical stimulation not under the control of the experimenter, or hypersensitivity to mechanical stimulation induced by inanition. In the last respect flies are like butterflies in that they sometimes become so sensitive during inanition that slight mechanical stimuli effect proboscis extensions, the proboscis even remaining permanently extended in some cases. Care was taken to avoid, in so far as possible, such sources of error, the animals being discarded if there was any regularly recurring question of interpretation. Occasionally, particularly with *Lucilia*, very slight movements of the oral lobes of the proboscis tip were noted which were apparently not coupled with any extensor movement. Such movements were counted as "no responses." Similarly, slight movements of the palp, noted in a few instances, were disregarded.

The methods used in this paper are similar to those used in previous papers dealing with similar problems. Each trial lasted 30 seconds, at the end of which period "no response" was recorded if no extension of the proboscis had been observed. Minimum intervals of ten minutes were allowed between trials. Extensions of the proboscis judged as less than one half of complete extension were weighted at .5, while extensions judged to be one half complete or more were weighted at 1. As a matter of fact the great majority of responses were complete extensions. The figures which will be chiefly discussed are the percents of weighted responses, called the *per cent. of response*, computed by dividing the total weighted response obtained in a series of trials by the maximum weighted response possible in the trials.

Experiments.

The questions to be answered by these experiments were two:

1. Do flies possess contact chemoreceptors in the tarsi similar to those present in butterflies?

2. If present, what are some of the substances discriminated by these sense organs?

It is easy to demonstrate that mounted flies when placed with their tarsi in contact with absorbent cotton saturated with distilled water or a 1 *M* saccharose solution will usually extend the proboscis. Such responses may conceivably be due chiefly or solely to: (1) tactile stimulation of the tarsi; (2) distance chemical stimulation of the antennæ or other receptors by water vapor; (3) contact chemical stimulation of the tarsi. Experiments were accordingly devised to test out these possibilities.

Two general types of experiments were devised, the first to test the ability of the tarsi to discriminate between water and paraffin oil; the second, to test the ability of the tarsi to discriminate between water and 1 *M* saccharose solution.

Three sets of experiments of the first type were carried out, alike as to object and general procedure but differing slightly in apparatus and methods. Under the general conditions of these experiments it was found that the great majority of flies had become 100 per cent. responsive to distilled water by the time trials were begun, viz., two to four hours after anæsthetization. A few individuals failed to become responsive in time to complete the experiment the same day, and still a few others failed to respond at all. Occasionally such animals were carried over to the second day and experimented upon, but, in general, they were discarded. The flies which did become responsive were given three trials with water, and alternating with these, three trials with oil. Great care had to be exercised in trials with oil, not to allow the body to come in contact with the oil saturated cotton and further, to remove the oil from the legs after each trial with shreds of filter paper. Otherwise the oil spread over the body and quickly killed the animal. With the proper precautions, however, flies could usually be given repeated trials with oil and yet be kept in good condition.

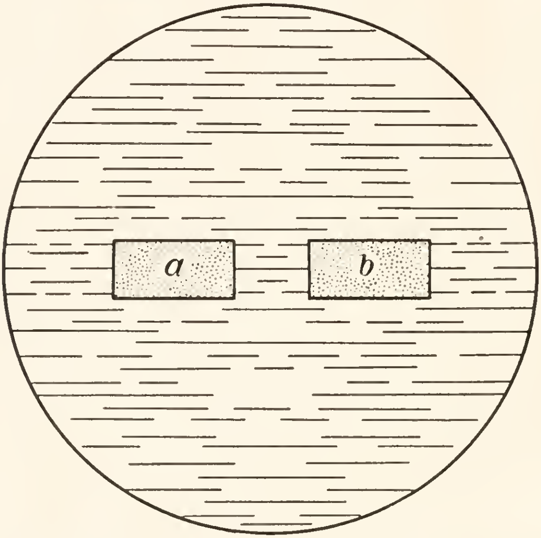

Fig. 2. Apparatus used in testing the effect of tarsal contact with water. The apparatus consisted of a petri dish filled with water. Near the center are two rectangular pans: *a*, containing a cotton pad saturated with distilled water, and *b*, containing a cotton pad saturated with paraffin oil.

The details of the first two sets of experiments were briefly as follows. To the bottom of a glass petri dish 14 cm. in diameter, two rectangular metal pans 2 cm. by 3.5 cm. by 1.5 cm. deep were fastened with wax. The petri dish was filled with distilled water nearly to the edge of the pans. In one pan (*a*, Fig. 2) was placed a smooth pack of absorbent cotton saturated with distilled water; in the other pan (*b*, Fig. 2) a similar pack, saturated with paraffin oil. The animals were tried in three positions: (1) with their feet in contact with the cotton of pan *a*, where they were subjected to the contact stimulus of water, and, the distance stimulus of water vapor; .(2) a similar position in pan *b*, where they were subjected to the contact stimulus of paraffin oil and the distance stimulus of water vapor; .(3) a position similar to 2, and far enough above pan *b* to prevent contact of the tarsi with the oil, in which position they were subjected to the distance stimulus of water vapor only. Positions 1 and 2 differed only in the nature of the contact chemical stimulus afforded the tarsi, the tactile stimuli afforded by water and paraffin oil and the distance stimulus from water vapor being nearly identical in the two cases. Position 3 differed from 1 and 2 in lacking any contact tarsal stimulation, either tactile or chemical.

TABLE I.

SHOWING THE RESULTS OBTAINED WITH TWO SPECIES OF *Phormia* UNDER THE FOLLOWING CONDITIONS OF STIMULATION: *A*, WATER VAPOR PLUS TARSAL CONTACT WITH WATER; *B*, WATER VAPOR PLUS TARSAL CONTACT WITH PARAFFIN OIL.

Number of Animals.		*A.* Water Vapor Plus Tarsal Contact with Water.	*B.* Water Vapor Plus Tarsal Contact with Paraffin Oil.
29 *Phormia;*[1] 6♂, 23♀	Total number of trials....	87	87
	Total number of responses	87	21
	Total weight of responses.	87	17.5
	Per cent. of response.....	100	20

[1] 4♂ and 19♀, *P. regina;* 1♂, *P. terræ novæ;* 1♂ and 4♀ belonging to these two species but not specifically identified.

The first set of experiments was carried out on twenty-nine individuals belonging to the two species of *Phormia*. The results (Table I.) showed 100 per cent. response in position 1, and but 20 per cent. response in position 2. The animals were not tried in position 3. In some of the trials in these experiments, however, a source of error was discovered. The cotton pad in the oil pan became accidentally contaminated with watei.

In the second set of experiments, carried out on *Lucilia sericata*, care was taken to avoid the sources of error in the first group of experiments. In position 1 (Table II) *Lucilia* gave 95 per cent. response, in position 2, 11 per cent., and in position 3, 13 per cent. These animals, therefore, actually responded more to water vapor than they did to water vapor plus contact of the tarsi with oil.

TABLE II.

SHOWING THE RESULTS OBTAINED WITH *Lucilia sericata* UNDER THE FOLLOWING CONDITIONS OF STIMULATION: *A*, WATER VAPOR PLUS TARSAL CONTACT WITH WATER; *B*, WATER VAPOR PLUS TARSAL CONTACT WITH PARAFFIN OIL; *C*, WATER VAPOR ONLY.

Number of Animals.		*A.* Water Vapor Plus Tarsal Contact with Water.	*B.* Water Vapor Plus Tarsal Contact with Paraffin Oil.	*C.* Water Vapor Only.
14 *Lucilia sericata;* 6♂, 8♀.......	Total number of trials...........	42	42	42
	Total number of responses.......	40	5	8
	Total weight of responses.......	40	4.5	5.5
	Per cent. of response.........	95	11	13

Because of the error involved in the first set of experiments with *Phormia*, another and third set was undertaken on these species. In these experiments the apparatus used in the first two sets was discarded for syracuse watch glasses containing cotton pads soaked in water or oil. The animals were tested: (1) on water soaked cotton, where they were subjected to tarsal contact with water plus water vapor; (2) on oil soaked cotton, where they were subjected to tarsal contact with oil (no water vapor); (3) over the water soaked cotton but not in contact with it, where they were subjected to the stimulus of water vapor only. The results (Table III.) show 100 per cent. response in position 1 (column *A*, Table III.) and but 5 per cent. response in position 2 (column *B*). After the trials in positions 1 and 2 were made, the animals were tested in position 3 where they gave 1.4 per cent. response (column *C*). In order to show that the experimental procedure had not rendered the animals less responsive, each animal was finally tested in position 1 again immediately after each trial in position 3. The result (column *D*) was 100 per cent. response. The low per cent. obtained in position 3 could not, therefore, be attributed to any changed condition in the animals.

The results of these experiments show two facts clearly.

TABLE III.

SHOWING THE RESULTS OBTAINED FROM TWENTY-FOUR FEMALES OF *Phormia regina* UNDER THE FOLLOWING CONDITIONS OF STIMULATION: *A*, WATER VAPOR PLUS TARSAL CONTACT WITH WATER; *B*, TARSAL CONTACT WITH PARAFFIN OIL; *C*, WATER VAPOR ONLY; *D*, SAME AS *B*. THE LAST SET OF TRIALS WAS MADE AS A CHECK.

Number of Animals.		*A.* Water Vapor Plus Tarsal Contact with Water.	*B.* Tarsal Contact with Paraffin Oil.	*C.* Water Vapor Only.	*D.* Water Vapor Plus Tarsal Contact with Water.
24 *Phormia regina;* ♀	Total number of trials...........	72	72	66[2]	58[3]
	Total number of responses.......	72	5[1]	10	58
	Total weight of responses.......	72	3.5	9	58
	Per cent. of response.........	100	5	1.4	100

[1] Confined to 3 of the 24 animals.

[2] Two of the animals were unfit for further experimentation after the first two sets of trials and had to be discarded, hence the number of animals here represented is only twenty-two.

[3] Trials in which water vapor called forth a complete extension of the proboscis could not be followed immediately by another trial. This explains why there were only 58 instead of 66 trials.

First, animals which gave about 100 per cent. response when their tarsi were brought in contact with water gave much less than 20 per cent. response when their tarsi were brought in contact with paraffin oil. Second, the response to water vapor alone was sufficient to account for at least part of the responses obtained with paraffin oil. In other words, in animals which were about 100 per cent. responsive to tarsal contact with distilled water, the response to contact with paraffin oil was very small. The response to the oil was not zero, however, as the results of the third set of experiments show. Since the tactile stimuli of water and oil are nearly alike, the great difference in response can only be due to the difference in the contact chemical stimulation afforded the tarsi. The tarsi of these flies must, therefore, possess contact chemoreceptors.

In the second type of experiment, distilled water and a $1M$ saccharose solution were used as stimuli. In these experiments syracuse watch glasses were used, each containing a layer of absorbent cotton, which was saturated with the solution to be tested. During the first phase of these experiments the flies were given neither water nor food. They were tested with distilled water until a complete extension of the proboscis was obtained in each of three successive trials. A few animals failed to meet this requirement and were discarded. The remainder were considered as standardized animals, 100 per cent. responsive to distilled water, and were subjected to further experiment.

Care was exercised in the first two trials with water to prevent the animals from drinking, but at the close of the third trial they were allowed to drink all the water they would. Beginning at this point, therefore, a period of water diet was initiated. Trials with distilled water were continued, each animal being allowed to drink each time it responded. On the average, however, the animals ceased to respond after two administrations. When no response had been obtained in three successive trials the animals were considered to have become zero per cent. responsive to water, a standardization which proved to be very accurate. The animals on which this experiment was completed were, therefore, selected animals which had successfully met two preliminary tests: (1) They had, after several hours without access to water, become 100 per cent. responsive to water; (2) they had upon administration of water, become 0 per cent. responsive to this stimulus. Out of 27 flies which had met the first test only 5, 3 *Phormias* and 2 *Lucilias*, failed to meet the second test in the time covered by the experiment.

For purpose of comparison, each time a "no response" to water was obtained, during the period of water diet, the animal was immediately tested with $1M$ saccharose. Following such trials the legs were carefully rinsed in distilled water to prevent contamination in future trials. In the trial with $1M$ saccharose following the third "no response" to water, each animal was allowed to drink all of this solution it would. Some individuals "drank" for only a few seconds while others remained with the proboscis extended for more than a minute. Thus, at this point

TABLE IV.

SHOWING THE RESPONSES OF STANDARDIZED FLIES TO DISTILLED H_2O AND 1*M* SACCHAROSE SOLUTION DURING PERIODS OF WATER DIET AND 1*M* SACCHAROSE DIET.

Number of Animals.		Period of Inanition.	Period of Distilled Water Diet.		Period of 1*M* Saccharose Diet.	
		Summary of Last 3 Trials with Water.	Summary of Last 3 Trials with Water.	Summary of Last 3 Trials with 1*M* Saccharose.	Summary of All 5 Trials with Water.	Summary of All 5 Trials with 1*M* Saccharose.
14 *Phormia*;[1] 6♂, 8♀....	Total number of trials....	42	42	42	65[2]	65
	Total number of responses	42	0	39	2?[3]	52
	Total weight of responses	42	0	39	2?	51
	Per cent. of response.....	100	0	93	3?	82
8 *Lucilia sericata*; 2♂, 6♀	Total number of trials....	24	24	24	35[2]	35
	Total number of responses	24	0	24	0	30
	Total weight of responses	24	0	23	0	28
	Per cent. of response.....	100	0	96	0	80

[1] 1♂ and 8♀. *P. regina*; 4♂, *P. terræ novæ*; 1♂ belonging to one of these species but not specifically identified.

[2] One animal failed to extend the proboscis sufficiently to bring it in contact with the 1*M* saccharose solution with the result that it could not be made to drink and could not be included in this part of the experiment.

[3] The responses here recorded, were in all probability not due to tarsal stimulation by water, but to the stimulation of the proboscis by regurgitated liquid. Both of these responses came from the same individual, and occurred under the same circumstances. The individual had been allowed to drink saccharose in the two trials preceding the trials in question. In each of the latter there was no sign of proboscis extension until nearly the end of the trial when a drop of clear fluid was regurgitated from the proboscis tip. A few seconds later the proboscis was extended and the drop sucked in. Since the tip of the proboscis is exceedingly sensitive to contact chemical stimulation, it seems almost certain that these responses are to be explained on this basis, rather than that of tarsal stimulation.

the period of water diet was ended and a period of 1 *M* saccharose diet begun. During this period each animal was subjected to five trials with water, each trial being followed, in case of no response, by a trial with saccharose. These experiments, therefore, furnish data on the response of animals which were 0 per cent. responsive to water, to 1 *M* saccharose during periods of water diet and 1 *M* saccharose diet.

The data from these experiments are to be found in Table IV. Both *Phormia* and *Lucilia* were employed, the results for the two species being strikingly similar. *Phormias* which had been rendered unresponsive to water by administration of the same, remained 93 per cent. responsive to 1 *M* saccharose, while *Lucilias* under the same conditions remained 96 per cent. responsive. When the diet was changed from distilled water to 1 *M* saccharose, the response to water continued to be zero or nearly so, and the response to 1 *M* saccharose, though somewhat diminished, continued to remain high: 82 per cent. in the case of *Phormia;* 80 per cent. in the case of *Lucilia*. Thus, the administration of water failed to reduce the response to saccharose appreciably, while the administration of saccharose reduced it somewhat.

Differences were noted not only in the numbers of responses under the various conditions described, but in the character of responses as well. In general, during water diet, the response to 1 *M* saccharose was much more vigorous than the response to water. The slightest contact of the tarsi with the sugar solution often caused the proboscis to be extended violently and to remain so until the solution was washed off. Corresponding to the diminution in the number of responses to saccharose during saccharose diet, there was also some lessening in the violence of the reaction. It is quite clear, therefore, that the contact chemoreceptors of the tarsi distinguish readily between water and a 1 *M* saccharose solution.

It has been previously stated that when tarsal stimulation with a sugar solution failed to elicit a response, contact of the oral lobes at the tip of the proboscis with the solution still proved an effective stimulus. There are, therefore, present on the oral lobes contact chemoreceptors, serving as organs of taste, which are even more sensitive than the organs of the tarsi.

These results on flies are similar to those obtained by the author on nymphalid butterflies (Minnich, '22*a*). In both cases the response to water can be completely controlled, while that to saccharose can not. In butterflies, however, the administration of 1*M* saccharose fails to alter the response to this solution at all while in flies, it causes a drop of about 20 per cent. In flies, further, the period required for the response to water to become 100 per cent. is measured in hours, while in butterflies it is measured in days. This is another indication of what is evident in many other ways, viz., that the metabolism and activity of the fly are much more intense than those of the butterfly.

Barrows ('07) observed that when one foot of a fruit fly touched food or a small drop of water the proboscis was immediately extended. He suggested that the two transparent hairs found beneath the claws of each front foot might contain the sense organs involved. B. M. Patten ('17) noted that the modified anterior pair of legs of the whip tail scorpion were sensitive to chemical stimulation including water. Similar observations showing the sensitivity of the walking legs to contact with food and other chemical stimuli in various Crustacea have been made by a number of investigators: Herrick (1895) for the lobster; Bell ('06) and Holmes and Homuth ('10) for the crayfish; and Balss ('13) for the prawn, Palæmon treillanus.

These observations together with the past and present observations of the author cover species from three classes of Arthropoda, viz., Crustacea, Insecta and Arachnida. The presence of contact chemoreceptors, serving as organs of taste, in the distal ends of thoracic legs must, therefore, be considered to be of general occurrence among arthropods.

General Summary and Conclusions.

1. The muscid flies, *Phormia regina* Meigen, *Phormia terræ-novæ* R.D., and *Lucilia sericata* Meigen, extend the proboscis upon appropriate contact chemical stimulation of the tarsi.

2. The response to water varies from 0 to 100 per cent., and the response to 1*M* saccharose from 80 to 100 per cent. depending upon the nutritional state of the animal.

3. By means of these reactions, it can be shown that the chemoreceptors in the tarsi serve as organs of taste.

4. These chemical sense organs can distinguish water from paraffin oil, and water from 1 M saccharose solution.

5. The oral lobes of the proboscis are also equipped with organs of taste.

6. The chemoreceptors of the oral lobes are more sensitive to 1 M saccharose than are the chemoreceptors of the tarsi.

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EFFECTS OF CHANGES IN MEDIUM DURING DIFFERENT PERIODS IN THE LIFE HISTORY OF *UROLEPTUS MOBILIS.*

LOUISE H. GREGORY.

2. THE EFFECTS OF DI-SODIUM AND DI-POTASSIUM PHOSPHATE·

In an earlier paper (1) results of experiments with *Uroleptus mobilis* were reported which indicate a variation in response to the use of a beef-flour medium according to the age of the protoplasm. Young and old individuals showed an immediate slowing of their rate of division while mature lines were definitely stimulated. With these results at hand it seemed worth while to continue the experiments to see if salts of various kinds would stimulate or depress at different age periods.

The material and methods used in conducting the experiments have been the same as those of the earlier work and as usual the rate of division is considered an indication of the vitality of the protoplasm. The salts used for experimentation have been di-sodium and di-potassium phosphate. The latter was found to be a stimulant to certain hypotrichs by Woodruff (2) in 1905, and the former caused an increase in the division rate of *Paramecium* (3) (Packard, 1926) and *Uroleptus* (4) (Austin, 1926).

Two types of experiments have been followed in using these salts, (1) experiments in which the individuals were subjected to an initial dose of the salt for thirty minutes, after which they were transferred to the normal hay-flour medium and treated thereafter in the same way as the control series for the following ten days; (2) experiments in which the salt was added to the normal medium daily in order to insure a constant effect.

A. The Effects of a Single Dose.

1. *Experiments with Di-potassium Phosphate.*

Sixteen experiments on thirteen different series have been conducted. In each experiment the individuals were placed in three drops of *N*/1000 K_2HPO_4 for thirty minutes and then were transferred to the normal medium. Table I. shows the results of these experiments, giving the age at the time of the experiment and the amount of variation from the control series of the number of divisions per line for the ten days.

TABLE I.

EFFECTS OF AN INITIAL DOSE OF DI-POTASSIUM PHOSPHATE ON PROTOPLASM OF VARYING AGES.

Series No.	Age in Gen.	Av. No. Divisions per Line in 10-day Periods, Experimental Series.	Av. No. Divisions per Line in 10-day Periods, Control Series.	Amt. of Variation in Division Rate per Line in 10-day Periods.
115	16	10.4	13.2	−2.8
129 *B*	18	7.4	13.0	−5.6
114	17	7.6	10.6	−3.0
113	23	7.4	8.4	−1.0
110	57	15.2	13.4	+1.8
133	63	13.8	11.0	+2.8
82	80	13.8	10.0	+3.8
81	156	9.6	9.2	+ .4
131	168	6.6	9.0	−2.4
133	192	11.8	9.6	+2.2
133	212	12.6	13.0	− .4
105	261	5.8	7.8	−2.0
131	280	9.6	7.8	+1.8
131	295	9.6	10.0	− .4
77	276	7.8	10.2	−2.4
69	284	10.0	10.4	− .4

In the first four experiments where the age of the protoplasm varied from the 16th to the 23d generation the treatment with the salt caused a lowered rate of division varying from one to three divisions per line less than that of the control series. The next ten experiments with lines whose age varied from the 57th to the 298th generation gave results which showed in six cases an increase in the division rate and in four a slight decrease. In no case was the change or variation from the control series very marked. In the last two experiments there was no stimulation of the vitality. The old lines were slightly depressed. Thus as in the experiments with the beef-flour medium, the young series are depressed by the potassium phosphate, the older lines too show a slower rate, while the intermediate individuals may be slightly stimulated by the treatment.

2. *Experiments with Di-sodium Phosphate.*

Two parallel experiments were carried on with di-sodium phosphate as the initial treatment. In one set a *N*/1000 solution of Na_2HPO_4 was used for the 30-minute initial dose, in the other set a *N*/7000 solution of the same salt was used. The latter strength was selected because it gives approximately the same percentage of sodium when added daily to the normal medium as that which caused a distinct increase in the rate of division of *Paramecium* (Packard) and of *Uroleptus* (Austin). Table II. gives the results of these experiments.

Table II.

The Effects of an Initial Dose of Di-sodium Phosphate on Protoplasm of Varying Ages.

Series No.	Age in Gen.	Av. No. Divisions per Line in 10-day Periods, Experimental Series.		Av. No. Divisions per Line in 10-day Periods, Control Series.	Amt. of Variation in Division Rate per Line in 10-day Periods.	
		N/1000.	*N*/7000.		*N*/1000.	*N*/7000.
129 *B*	18	11.6	8.8	13.0	−1.4	−4.2
133	63	13.6		11.0	+2.6	
133	74		11.0	13.8		−2.8
131	165	10.2		9.7	+ .5	
131	174		16.4	13.6		+2.8
133	192	9.8		7.0	+2.8	
133	200		11.4	9.6		+1.8
133	212	12.0	13.2	12.0	+0.0	+1.2
131	280	6.2	7.2	7.8	−1.6	− .6
131	295	11.8	9.4	10.0	+1.8	− .6

At this time series 129 *B* was the only young series available for experimentation. This series was in the 18th generation and after the treatment with both the stronger and weaker solution, the division rate was lowered 1.4 divisions per line in the one case and 4.4 divisions per line in the other case as compared with the control series. The other nine experiments with individuals that varied in age from the 63d to the 295th generation showed the varying results that are characteristic of mature protoplasm, the majority being stimulated by the treatment. A few were slightly depressed and there was but little difference in the effects of the two solutions.

These results when considered alone are too few to allow any definite conclusions to be drawn, but when they are considered in the light of the preceding experiments with potassium phosphate and previous ones with beef-flour they indicate the same variations in the response of the protoplasm at different ages.

B. THE EFFECTS OF DAILY STIMULATION WITH DI-POTASSIUM AND DI-SODIUM PHOSPHATE.

1. *Experiments with Potassium Phosphate.*

Initial experiments were conducted with series 133 at a period of early maturity in the 104th generation in order to determine the most favorable amount of K_2HPO_4 to be added to the normal medium. Five series of five lines each were kept for ten days in a medium consisting of normal hay-flour mixture to which potassium phosphate in different densities was added. The results showed that the individuals kept in a medium to which had been added daily $N/5000$ K_2HPO_4 gave the highest division rate averaging 5 divisions per line more than that of the control series. This strength of solution therefore was used in all of the experiments with potassium phosphate. Table III. shows the results of these experiments.

TABLE III.

EFFECTS OF POTASSIUM PHOSPHATE ADDED *Daily* TO NORMAL MEDIUM.

Series No.	Age in Gen.	Ave. No. Divisions per Line in 10-day Periods, Experimental Series.	Ave. No. Divisions per Line in 10-day Periods, Control Series.	Amt. of Variation in Division Rate per Line in 10-day Periods.
131 *B*	20	14.6	16.0	−1.4
129 *B*	58	14.8	16.0	−1.2
129 *B*	80	14.6	10.2	+4.4
133	104	20.6	15.6	+5.0
133	122	18.8	16.6	+2.2
133	192	14.8	7.0	+7.8
133	212	14.6	12.0	+2.6
131	220	15.8	15.6	+ .2
131	280	11.8	7.8	+4.0
131	300	9.6	9.2	+ .4
128	300	8.6	8.8	− .2

Two young series 131 *B* and 129 *B* in the 20th and 58th generation respectively were transferred daily for ten days to the potassium-hay-flour medium. Both series as a result of the treatment showed a lowered vitality and a slower division rate than the control series. In the 80th generation however, 129 *B* was definitely stimulated when again treated daily with the potassium-hay-flour medium, dividing 4.4 divisions per line more than the control series. Series 133 was treated in the same way in the 104th, 122d, 192d, and 212th generation for ten days each and at each period the division rate was increased over that of the control.

Series 131 was treated three times with the potassium-hay-flour medium. In the 220th generation it was but slightly stimulated during the ten days in the medium. In the 280th generation the division rate rose to 4 divisions more per line than the control series and finally in the 300th generation, the rate dropped to but .4 divisions per line more than the control series. This was a vigorous series throughout all of the experiments and probably the usual old age reaction was only beginning in the 300th generation. As has been clearly shown by Calkins (5) in his study of this species, the various series differ in the length of life and also in general vitality. Some series live a long life with a moderate division rate which diminishes gradually during old age, others have a high division rate, respond definitely to changes in the environment and then die out quickly. Series 133 seems to have been more like the latter type while series 131 always showed a more moderate division rate which was less easily changed by variations in the environment.

The oldest series (128) in the 295th generation showed a slight depression in its vitality when treated with the potassium-hay-flour medium. These results seem again to indicate that potassium used as a constant addition to the normal medium, causes the same changes in vitality as when it is used as an initial dose; the youngest lines are depressed, the mature ones are stimulated while the old lines are only slightly effected if at all.

The hydrogen ion concentration of the potassium-hay-flour medium used in all the above experiments was 7.2 which is that of the normal medium. When testing for the most favorable

TABLE IV.

EFFECTS OF VARIATION IN HYDROGEN ION CONCENTRATION OF POTASSIUM-HAY-FLOUR MEDIUM ON PROTOPLASM OF DIFFERENT AGES.

Series No.	Age in Gen.	Av. No. Divisions per Line in 10-day Periods, Distilled Water Solution, pH = 6.8.	Av. No. Divisions per Line in 10-day Periods, Spring Water Solution, pH = 7.2.	Av. No. Divisions per Line in 10-day Periods, Control Series.	Amt. of Variation in Division Rate per Line in 10-day Periods.	
					Distilled Water Solution.	Spring Water Solution.
131 *B*	20	12.2	14.6	16.0	−3.8	−1.4
129 *B*	58	13.6	14.8	16.0	−2.4	−1.2
133	122	16.8	18.8	16.6	+ .2	+2.2
131	229	14.0	15.8	15.6	−1.6	+ .2
128	295	5.8	8.6	8.8	−3.0	− .2

amount of K_2HPO_4 to be added to the normal medium, as mentioned above, the pH was taken of the different solutions and found to vary from 7.8 in the medium plus $N/1000$ K_2HPO_4 to 7.1 in the medium plus $N/6000$ K_2HPO_4, and the greatest division rate was found among the individuals living in the medium to which had been added a solution of $N/5000$ K_2HPO_4 and which showed a pH of 7.2.

The results of experiments in this connection are shown in Table IV. Two experiments were conducted with five series of different ages. The experiments were identical except for the fact that in one the potassium solution was made up with distilled water and added to the normal medium, which then had a pH of 6.8, while in the other experiment the potassium solution was made as usual with spring water and the resulting medium showed the normal pH of 7.2. In every experiment the distilled water series showed a lower division rate than that of the spring water series and also than that of the control series except in one instance. The youngest series 131 *B* in the 20th generation divided 3.8 divisions less per line than the control series and the oldest series 128 in the 295th generation was also depressed and divided 3. divisions less per line than the control. Series 133 in the 122d generation was the only series that was able to live in the unfavorable medium without showing depression. Here the protoplasm was in the early stage of maturity and apparently could adjust itself more easily to the changed conditions. Undoubtedly an acid condition creates an unfavorable environment in which the youngest and oldest individuals are unable to maintain their normal activity. The method of distillation of the water may be an additional factor in causing depression. However, when the solutions are made with spring water and have the same hydrogen ion concentration as has been the case in all the experiments with this one exception, some other explanation must be sought to explain the variation in the response of protoplasm of different ages.

2. *Experiments with Di-sodium Phosphate.*

In all of the experiments in which *Uroleptus mobilis* was kept in a medium to which sodium phosphate was added, the amount of sodium used was that found by Packard to cause a definite increase in the division rate of *Paramecium.* The same amount was also used by Austin in her experiments with the life history of *Uroleptus.* This amount was approximately a $N/7000$ solution of Na_2HPO_4 which was added to the spring water and used in diluting the hay-flour medium.

Eleven experiments were carried out for ten day periods with protoplasm of different ages and the results are shown in Table V.

TABLE V.

EFFECTS OF SODIUM PHOSPHATE ADDED *Daily* TO THE NORMAL HAY-FLOUR MEDIUM.

Series No.	Age in Gen.	Av. No. Divisions per Line in 10-day Periods, Experimental Series.	Av. No. Divisions per Line in 10-day Periods, Control Series.	Amt. of Variation in Division Rate per Line in 10 Days.
129 *B*	18	16.6	13.0	+3.6
131 *B*	40	15.2	10.0	+5.2
129 *B*	80	14.4	10.2	+4.2
133	90	21.4	17.0	+4.4
133	144	16.4	7.6	+8.8
131	179	19.7	16.0	+3.7
133	192	13.6	7.0	+6.6
133	212	14.8	12.0	+2.8
131	245	22.8	10.8	+2.0
131	280	10.4	7.8	+2.6
131	295	11.8	9.2	+2.6

In every experiment regardless of the age of the series there was an increase in the division rate. There was however, a marked difference in the amount of increase according to the age of the protoplasm. The youngest series, 129 *B* in the 16th generation, was stimulated to divide 3.8 divisions more per line than the control series. In the 80th generation the same series increased its division rate to 4.2 divisions more than the control series. Series 131 *B* in the 40th generation was 5.2 divisions per line ahead of its control series. Series 133 in the 90th generation exceeded the control series by 4.4 divisions per line, in the 144th generation by 7.4 divisions per line, in the 192d generation by 6.6 divisions and in the 212th generation by 1.8 divisions per line. Series 131 was stimulated in the 179th generation and showed a division rate of 3.1 divisions ahead of its control series.

In the 245th generation the increase over the control fell to 2. divisions per line above the control and maintained this same variation when stimulated in the 300th generation. Thus in every case the sodium phosphate when a part of the normal medium caused an increase in the vitality of the protoplasm but the amount of the increase varied, being greatest in the height of maturity and less in the period of youth and least in the commencement of old age.

Conclusions and Summary.

The foregoing experiments seem to indicate a variation in the response of *Uroleptus mobilis* to a single treatment with salts or to a constant addition of salts to the normal medium. Di-potassium and di-sodium phosphate when used as an initial dose and di-potassium phosphate when added daily to the normal medium cause a distinct depression of the vitality of the young series, in general a stimulation of the more mature individuals, and a slight depression as the protoplasm increases in age. These results agree in the main with those from the beef-flour treatment. If the hydrogen ion concentration of the medium falls much below that of the control, the depressing effects are more marked in both the young and old series and the stimulating effect on the immediately mature individuals is almost obliterated. The increase in acidity may not have been the only cause of the depression as Hartmann (6) found the method of distillation of the water used in his experiments to be an important factor in maintaining a normal culture of *Eudorina elegans*. When sodium phosphate is used as a constant stimulation there is a general increase in the division rate of all ages, but that exhibited by the young and ageing protoplasm is markedly less than that seen in the intermediate lines. The sodium must increase the permeability of the cells but if this were the whole explanation we should expect to have approximately the same increase in division rate for all the individuals. There seems to be a weakening of the power of adjustment in both the young and old series, in the former possibly the derived organization has not yet become established and in the latter there is possibly an increasing stability or a loss in lability of the protoplasm with advancing age.

The complete histories of several series showing their response to treatment at different periods in the life cycle would indicate clearly this variation in the activities of the protoplasm. Further experiments are planned to emphasize this point showing the instability of youthful protoplasm, the gradual increasing ability of maturing protoplasm to adjust itself to changes in its medium and finally the loss of this power as old age advances.

These experiments with potassium and sodium phosphate using series of different ages as well as the same series at different periods in its life history, add further evidence to the theory of Calkins (5), that changes are taking place in the derived organization of the protoplasm of *Urolcptus mobilis* throughout the life cycle. These results can not be explained as ordinary variations in the division rate as the numbers of experiments are too many and the results too uniform; neither can they be due to variations in the hydrogen ion concentration as that has been maintained practically the same as that of the control save in the one experiment. Under certain conditions a low hydrogen ion concentration may be a secondary cause of the variation in response, but the primary, underlying cause must be sought in the condition of the protoplasm itself, its organization changing as the series passes from the period of youth or immaturity, through one of maturity and finally into that of old age.

Barnard College,
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ON THE STERILIZATION OF *DROSOPHILA* BY HIGH TEMPERATURE.

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Breeding experiments with *Drosophila melanogaster* at high temperatures have demonstrated that at a point 5° C. to 9° C. above the normal optimum of 24° C. no offspring are produced after one generation. This fact was first noted by Northrop (1) for a single stock. Plough and Strauss (2) showed that the exact temperature at which complete sterilization occurred varied with different stocks, but was approximately constant for any particular one. In all cases a temperature was found which produced complete sterilization after an exposure of four days or more, even though adult flies continued to live for some time at this temperature, and newly laid eggs developed, pupated and went through metamorphosis normally enough. The additional fact was noted by all these workers that flies thus sterilized would usually recover normal fertility if they were replaced for a day or two at a temperature of 24 degrees. Northrop stated however: "If after they have emerged from the pupæ they are left longer than ten days at the higher temperature the injury becomes permanent and they are no longer able to produce eggs capable of development at any temperature."

Attempts to explain this sterility in terms of effects on the germ cells have not been entirely successful. Northrop stated, though without crucial evidence, that "Imagos raised and kept permanently at a temperature of 30 degrees, are unable to produce eggs capable of development at this temperature." Plough and Strauss demonstrated that this could not be the correct explanation by crossing both males and females hatched at 31 degrees to females and males respectively bred at 25 degrees, carrying the crosses at 31 degrees. Most of the 31 degree females mated to normal males gave offspring at 31 degrees, and about half of the 31 degree males likewise. The actual figures summarized from Plough and Strauss, Table II., are:

	Fertile Cultures.	Sterile Cultures.
31° ♀ × 25° ♂	13	2
25° ♀ × 31° ♂	6	5

In spite of the excess in the number of fertile cultures from females this result was taken to indicate that "neither the eggs nor sperm are injured by exposure to 31° C." Cytological examination showed that apparently normal eggs and sperm were present in flies sterilized by heat, and copulation was observed between these flies at the high temperature. Plough and Strauss thus summed up the matter: "The reason for the failure to produce offspring after the first generation seems to lie either in a failure of the sperm to reach the eggs,—that is unsuccessful copulation—or in the failure of the fertilization process itself."

Further investigation of the question was delayed until the winter of 1924–5 when one of us—Young—was making a series of tests designed to determine whether strains of flies showing differing degrees of toleration of high temperature could be isolated from a single mass culture of a wild stock. The results of this series of tests will be reported elsewhere, but the experiments offered an excellent opportunity to settle the question raised above.

An examination of the Plough and Strauss data quoted above shows only that eggs and sperm are not injured by exposure to 31 degrees in every case. Sterile cultures did occur, and a comparison of the relative numbers suggests, in addition, that males are more likely to be sterilized than females. Since the 31 degree flies used were not actually tested and shown to be sterile at 31 degrees, but were taken from susceptible stocks (*i.e.*, stocks which ordinarily failed to reproduce at 31 degrees), it is possible that some of the flies which gave offspring in crosses might have been fertile even if they had been mated inter se at 31 degrees. For these reasons we returned to the question of the possible injury to the germ cells of the flies by high temperature, and made more extended breeding tests, and more careful cytological examination of the gonads from flies sterile at 31 degrees. Most of the breeding tests were made by Young, while the remainder together with the cytological study were made by Plough.

EXPERIMENTS.

The *Drosophila* stocks used for the tests were for the most part taken from a wild stock culture collected at Canton, Ohio, in September 1924, but for certain of the experiments other stocks were used as indicated. They showed a varying degree of toleration of high temperature as recorded by Plough and Strauss, but even though the critical temperature was different for different stocks each could be rendered sterile at some particular temperature. Even when a culture showed sterility it was observed that copulation between the flies takes place, and that eggs were laid in numbers roughly equal to those from similar flies at normal temperature. Since these eggs appear to be normal and since virgin females are known to lay eggs under normal temperature conditions, the conclusion is again suggested that the sterility at high temperature is caused by some failure to function on the part of the males.

Further data similar to those of Plough and Strauss were first collected. A series of lines of Canton stock were being run at 31 degrees. Since this temperature is close to the limit at which this stock can be bred, it was found that several strains usually showed sterility in each generation. There was a high degree of constancy in the reaction to temperature of these strains, some failing regularly after one generation, others running along for several generations normally. Matings were made at 31 degrees between both males and females from a number of these lines and flies of the opposite sex from the same strain carried continuously at 24 degrees. Normal flies of this stock are always fertile for at least one generation at 31 degrees. If a culture of the 31 degree stock showed sterility, then it might be supposed that the mating of sibs of this strain to the 24 degree stocks would indicate whether one sex rather than the other was responsible for the failure. The cultures in all cases contained five pairs of flies each, and a summary of the tests is given in Table I.

A comparison of the totals in this table emphasizes the suggestion of the summaries of the similar tests of Plough and Strauss, namely that most of the males from a culture at 31 degrees are sterile, while most of the females are fertile. This

Table I.

Tests Run at 31 Degrees.

Controls (Sibs of Tested Flies at 31°).	♂ Hatched at 31° × ♀ at 24°.		♀ from 31° × ♂ from 25°.	
	Sterile.	Fertile.	Sterile.	Fertile.
Fertile—16	8	8	0	16
Sterile—12	11	1	6	5
Total Cultures	19	9	6	21

becomes still more significant when the figures are examined in comparison with the corresponding controls. Where these were fertile, all of the females were fertile, yet one half of the males were sterile. Apparently the males of a culture begin to be sterilized by heat, before there is any effect on the females whatever. When the controls were sterile, in only one out of twelve cultures did the males show fertility, while about half of the females are affected and half are still fertile. It would appear that high temperature causes at least partial sterilization of the males at a point where the females are largely unaffected. Eventually at least some of the females are sterilized. It was noticeable that in the strains showing a high degree of tolerance for 31 degrees, the males only showed sterility.

In order to establish these facts beyond question it seemed wise to run a more extended series of matings to normal stock using cultures each of which was actually known to be sterile at high temperature. A series of cultures of flies of different strains was placed in the incubator at 32 degrees. This is sufficiently high to cause sterility in nearly all strains after the first generation, and in every one of the cases tabulated this was the result. Each fly was then mated to another of the opposite sex which had been hatched at the optimum temperature of 24 degrees, and all were replaced at 32 degrees. The results of these tests are given in Table II.

The totals show that from cultures which gave no offspring at high temperature, 96 per cent. of the males were actually sterile, while only 50 per cent. of the females were affected. More detailed analysis makes plain the fact already demonstrated by

Table II.

Flies Sterile at 32°. Remated and Continued at 32°.

The numbers given refer to individual flies.

Stocks.	♂ from 32° × ♀ from 25°.		♀ from 32° × ♂ from 25°.	
	Sterile.	Fertile.	Sterile.	Fertile.
(1) Porto Rico	12	0	0	16
(2) Canton	3	0	0	3
(3) Amherst	8	2	8	4
(4) Prague	12	0	6	3
(5) Brown	6	0	6	0
(6) Spineless	6	0	6	0
Totals	47	2	26	26
%	96%	04%	50%	50%

Plough and Strauss, by Strauss (3), and by the unpublished work of Young mentioned above, that stocks show differences in their reactions to high temperature, some being much more tolerant than others. In the most tolerant stocks—Porto Rico and Canton—there is a clear differential effect such that the males are rendered completely sterile while the females are unaffected. In less tolerant stocks like the Prague and Amherst cultures the females show some effect, though it is not so great as in the case of the males. In mutant stocks like spineless and brown there is much less resistance and both sexes are rendered completely sterile. From these and other data there is evidence that at a temperature somewhat lower than 32 degrees even these stocks show the same differential sterilization of the males without any effect on the females.

Turning now to experiments designed to test the length of time required for flies sterile at 31 degrees to recover fertility when returned to 24 degrees, we find this selective effect of high temperature on the male flies again clearly demonstrated. This recovery of fertility—first noted by Northrop as indicated above—was shown by removing cultures from the 31 degree incubator at intervals and placing them in new bottles at 24 degrees. Controls were furnished by cultures of normal flies bred continuously at 24 degrees, and also by females from the sterile 31 degree cultures mated to normal males. In all cases the bottles contained five pairs of flies each, so that individual differences are largely eliminated from the result. The time of the appearance of the first eggs and first larvæ are noted in each case, and the data are tabulated in Table III.

Table III.

Canton Stock Cultures Sterile at 31°, Removed and Placed at 24°.

Description.	No. Days at 31° after Hatching.	No. Cultures.	Average No. Days before Eggs Appeared.	Average No. Days before Larvæ Appeared.	No. Cultures Failed to Recover.
Flies bred at 24° continuously	0	17	1.5	2.5	—
Females from sterile cultures × males bred at 24°	10 (♀ only)	7	1.5	3	0
Sterile 31° cultures	2	4	1.5	7.5	0
" " "	6	8	1.5	7.5	1 (12½%)
" " "	10	6	1.5	9	1 (16.6%)
" " "	13	13	1.5	9	5 (38.4%)

For the cultures recorded in Table III. the date of the first adult flies was recorded also, but is not noted in the table since the average number of days between the appearance of larvæ and the emergence of imagos was the same in all cases—about six days. The second line in the table affirms the result given in Tables I. and II., namely that females are not affected by a temperature of 31 degrees and their eggs develop as soon as fertilized. The remainder of the table therefore indicates the effect of the high temperature on the male flies. The females begin to lay eggs at once, but they do not lay eggs which develop until a period of seven to nine days has elapsed. The time when fertile eggs begin to be laid bears only a slight relation to the length of exposure to high temperature after hatching, but the last column showing the number of cultures which failed to recover, indicates that more males never regain fertility after a longer exposure.

The experimental data apparently demonstrate that high temperature has a differential action on the germ cells of *Drosophila* such that males are rendered completely sterile at a point at which females are uninjured. This effect is permanent as long as the temperature is maintained, but male flies will recover normal fertility if returned to the optimum temperature for a variable period. Longer exposures, however, tend to greatly increase the number of male flies which are permanently sterilized. Apparently raising the temperature still higher eventually sterilizes the females as well, and finally kills the flies.

Cytological Observations.

Sections of testes and of ovaries from flies which failed to produce offspring at 31 degrees were made, and compared with similar sections from normal flies of the same age. In addition the spermathecæ and ventral receptacles of females of the two sorts were examined both whole in Ringer's solution and in stained sections.

Study of these preparations adds very little to the facts already shown by experiment, though it confirms these. In none of the sections is it possible to discover any difference in the cytological picture between normal ovaries and ovaries of females from sterile cultures, except that the latter appear to be smaller, with fewer eggs at similar stages. Sections of the widened end of the testis just about at the point where it joins the vas deferens were studied in a sterile male and in a normal male respectively. The testes were taken from flies one group of which had been hatched and kept at 31 degrees for twelve days and the other at 24 degrees for the same time. The spermatozoa in the latter completely fill and distend the surrounding membrane. The same region in a sterile male is partially collapsed, and contains only a few scattered sperm. In other sections even these are absent. In portions of the testis slightly further up one can see a few cells which are probably spermatocytes together with some spermatozoa. At about the same place in testes from sterile flies only masses of spermatozoa appear. These seem to be shrunken away from the wall in masses and appear to be undergoing degeneration for neither their size nor arrangement is normal. In other sections from sterile males degeneration is also suggested for the sperms are decidedly reduced in number, have lost their orderly arrangement in masses in the testis, and seem shrunken in compact irregular aggregations. Apparently a few pass down into the vas deferens but these are probably not functional.

Ventral receptacles of females from sterile and fertile cultures respectively were also examined. Actual dissection of such flies in Ringer's solution shows clearly that spermatozoa are present in the receptacles of normal flies, but they can not be found in the females from sterile cultures. The sections simply confirm these findings. Masses of sperm can be seen clearly to fill the lumen of the receptacles of flies from normal cultures while ducts of flies from sterile cultures are empty. These observations prove that even though the male and female flies of sterile cultures copulate no transference of sperm to the receptacles of the females takes place.

Beyond these facts cytological study reveals little. The testes of sterile flies are smaller on the average, and there is some suggestion of disorganization in the cells in the lower portion, but this is not constant. However it has been shown above that exposure of the adult males to 31 degrees for ten days or more produces sterility from which a certain number do not recover when they are placed at 24 degrees. If only the spermatozoa are affected, it is hard to see why this should happen. The data showing the recovery of the male flies suggests a progressive sterilizing effect beginning with the sperms and eventually reaching cells at earlier stages. Only when the latter occurred would the male fail to recover fertility when placed at 24 degrees.

It is interesting that one pair of testes greatly reduced in size was found in a sterile male and sectioned separately. Both testes appear to have been but empty shells for neither spermatozoa nor any clear germinal tissue whatever were present. This may have been a degenerative change produced by high temperature, but such testes occasionally appear in normal stock. None did appear among the controls examined, however.

The cytological observations therefore confirm the breeding tests by showing that a temperature of 31 degrees sterilizes male flies, with little or no effect on the females. The sterility appears to be caused by some degenerative effect on the spermatozoa, such that all become non-motile, and eventually may be killed. Recovery apparently consists in the formation of spermatozoa anew from cells in the growth period or earlier.

Discussion.

These facts are of especial interest when considered with others recently recorded. Cells of the male germ line are here shown to be much more sensitive than eggs or other tissues to a rise of 6 or 7 degrees in a cold-blooded animal, the fruit fly. C. R. Moore (4) has given similar evidence of the sensitiveness of the male germinal epithelium in warm blooded animals in his extensive studies on experimental cryptorchidism in mammals. He finds that when testes are exposed to the higher temperature of the peritoneal cavity rather than the scrotum there follows a rapid and progressive degeneration of the germinal cells. Such testes show progressive recovery when returned to their normal position. Bluhm (5) has given interesting evidence of the greater sensitivity of spermatozoa of mice to drugs (alcohol, caffein, etc.) since a portion of one class of spermatozoa, the female producing, become non-functional. Further the recent tests of Mavor and DeForest (6) on the sensitivity of eggs and sperm of *Arbacia* to X-rays show a much greater sterilizing effect on the sperms at all doses. Evidently the male germ cells are much more susceptible than eggs or other cells of the animal body to external influences.

Summary.

1. High temperature has a differential effect on the germ cells of *Drosophila* such that males may be rendered completely sterile at a point at which females are still completely fertile.

2. This effect on the males is permanent as long as this temperature is maintained, but most males will recover normal fertility after being returned to the optimum temperature.

3. Exposures to 31° C. of over ten days tend to increase greatly the number of flies which are permanently sterilized.

4. Examination of the testes of sterile males shows that high

temperature causes loss of motility of the sperm, with progressive aggregation and degeneration. Few or no sperms are found in the lower end of the testis and the vas deferens.

5. Although such males copulate with females at high temperature, no sperms pass into the ventral receptacles of the females.

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ON THE LUMINESCENCE OF *MICROSCOLEX PHOSPHOREUS* DUG.[1]

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In spite of several papers describing observations on the luminescence of different species of earthworms, there are still some uncertain points which deserve further investigation. In Mangold's ('14) article, which discusses thoroughly the power of luminescence in all groups of plants and animals, the conclusions lead to the assumption that the production of light in these animals is due to infection by luminous microorganisms or fungi, which may be present in places where earthworms obtain their food. Dubois ('14) and Linsbauer ('17) were unable, however, to show in the luminous slime any luminous microörganisms, which is a strong argument against the view of the bacterial origin of luminescence in this group of the animal kingdom. Gilchrist ('19) working on the South African earthworm species *Chilota*, describes many observations and experiments which seem to show that the luminescence in this species is not bacterial. Quite recently, however, Pierantoni ('24), according to his "hereditary symbiosis theory," believes that *Microscolex phosphoreus* owes its luminescence to symbiotic bacteria living inside the cells of its body. It is the purpose of this paper to show how the luminescence in *Microscolex* originates, and which opinion rests upon a sounder basis.

OBSERVATIONS AND EXPERIMENTS.

Microscolex phosphoreus,[2] a small earthworm with clitellum situated near the anterior part of the body is easily to be found in Naples and its environs. If the animal be kept undisturbed, one cannot observe the production of luminous slime, which is discharged only on mechanical, chemical or electrical stimulation. It may be mentioned here that all the specimens of *Microscolex phosphoreus* examined by me were found to be luminescent. In free living animals the small hard particles of the soil may produce, by irritating the skin, the necessary stimulus for the secretion of the slime, which glows brightly with a yellow-greenish tinge. The slime is usually ejaculated from the anal aperture, and only in some specimens kept previously for a longer time undisturbed, did the slime flow also from the mouth opening. The posterior and the anterior end of the alimentary canal were the only two points at which the production of luminous slime was observed. As all these observations were carried out under the binocular microscope it is quite certain that the glands of the skin and the excretory system do not take part in the secretion of luminous slime. If the animal be wounded, however, on any point of the body, the luminescent slime flows from this part, the clitellum not being an exception. Nevertheless the luminous material does not arise from the alimentary canal, because in such cases the luminescence appears when the wound has not reached the intestinum. An injury of the body cavity is sufficient in itself for a production of luminous slime, which does originate, therefore, from the cœlomic fluid. This fact was corroborated by microscopical observations. A freshly discharged slime is composed chiefly of round cells with many, strongly light-refracting granules, scattered inside the protoplasm. Cells of this kind are found abundantly in the body cavity. Besides the uninjured cells, a certain amount of free granules, liberated by breaking of the cells, is always suspended in the fluid. I wish to call attention to the fact that both the size of those cells, and the structure of the intestinum, excludes the possibility of any other way of reaching the alimentary canal by the cells, except by preformed openings at its posterior and anterior end. That the communication with the body cavity is possible in *Oligochæta* was also shown by Gilchrist in *Chilota*, though in *Microscolex* the posterior rather than the anterior part of the intestine is the chief point at which ejaculation of the luminous slime occurs. Benham ('99) found that in the New Zealand earthworm, *Octochætus multiporus*, the cells of the luminous slime take their origin from the body cavity. The fluid is discharged, however, "from the dorsal pores and from the mouth, which it reaches through the peptonephridia opening into the buccal cavity." These observations show clearly that it is most improbable that the granules alone (Pierantoni's bacteria) may pass through the connective tissue and two layers of muscular system until they reach the glands of the skin as Pierantoni seems to believe.

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[1] This work was done while holding the International Education Board Fellowship.

[2] I wish to thank Mr. G. E. Hutchinson for his help in identifying the earthworms.

The next task was to find out whether the cells or the granules alone have the power of luminosity. To test this the luminous extract, obtained by grinding up the whole animals, which easily passes through ordinary filter paper without loosing its luminescence, was centrifuged for 15 min. The centrifuged fluid in which only granules were seen could be made luminous on addition of a few drops of ether. After filtration through bacteriological filters, however, no light was observed upon treatment with ether. The granules therefore must be regarded as a luminous material in *Microscolex*. This agrees with Gilchrist's observations on *Chilota*.

It was noticed that the power of the ejaculation of the slime is directly proportional to the degree of the irritability of the nervous system. Luminous microörganisms, however, glow continually, and are not affected by nerve stimulation when living in luminous organs of some other forms (in certain fishes and cephalopods). A lowered temperature also affects the luminescence, probably by the lessened irritability which is especially noticeable if the animals be kept for a long time at 8°–10° C. In connection with this I should like to mention the day-night rhythm of luminescence in *Microscolex*. It was observed that if stimulated, the animal discharged more easily the luminescent slime during the night than in the daytime. It can be shown that this rhythm does not result from an inhibition by light, because the strong arc lamp and sunlight have no visible effect on luminescence. It is possible that this rhythm may be explained by the general lower irritability of the animals during the daytime.

Before the characteristics of the luminescence will be discussed, it must be stated that if the properties of the luminous slime be compared with those of an extract from the ground worms, it is easy to find a very striking difference. The slime continues to glow for about half an hour if kept moist, while on the contrary the light of the extract dies quickly, and on addition of water, immediately. Filter paper saturated with slime gives off light after an hour and a half if moistened; the dried extract, however, prepared from the ground worms, does not show any luminescence. It is difficult to explain these facts by oxydation of luminous material alone, especially as the experiments of Gilchrist show that pure luminous slime of *Chilota* mixed with water glows brightly for over two hours. Acidity does not seem to play an important rôle in the above mentioned observations, as solutions with different pH did not prolong the luminescence. It seems more probable that this difference may be due to some enzymes liberated from the cells by grinding, which have an inhibiting effect upon the luminous material.

If to luminous bacteria some chloroform or ether be added, the light becomes fainter and dies out quickly. On the contrary the extract from certain luminous animals such as *Pelagia* gives a bright flash of light on addition of fresh or distilled water, ether, saponin, etc. (E. N. Harvey, A. R. Moore). The extract of *Microscolex* reacts like that of *Pelagia*. Of the cytolytic substances tried—ether, chloroform, saponin, sodium glycocholate—only ether gives positive results. The light was however never as bright as in the living animal, but still visible. Owing to the rather small size of the animals it was difficult to experiment with pure luminous slime, but a few observations showed that in this case the action of the cytolytic substances were more clearly seen. Ether and chloroform produce the brightest luminescence of luminous slime of *Microscolex* and the same effect was observed by rubbing it with a finger.

E. N. Harvey supposes that the action of cytolytic substances may cause the cyto- or granulolysis of luminous bodies. In *Microscolex* it was difficult to observe directly the changes in the granules themselves, but after a longer time the slime treated previously with ether shows swelling of the luminous granules.

It may be remarked that NaF in 1.5 per cent. concentration has no inhibiting effect on luminescence of the slime or the extract of *Microscolex*. The light of free living luminous bacteria, however, ceases quickly in this solution and the same was observed by E. N. Harvey on the symbiontes multiplying in the luminous organs of the two fishes, *Photoblepharon* and *Anomalops*, and by me in *Sepiola intermedia*.

The presence of oxygen is necessary for the luminescence of *Microscolex*. It is obvious that the behavior of the earthworms does not differ on this point from many luminous organisms such as *Cypridina*, firefly, bacteria and others. In an atmosphere of hydrogen the luminescence disappears quickly and does not return on mechanical or electrical stimulation. After removing the animals from hydrogen the luminescence appears again. The luminous slime kept on a slide or the filter paper impregnated with slime glows brightly upon moistening with water even after an hour and a half. After this time no light can be observed. Under the cover-glass however, if the edges be covered with vaseline, the light appears after many days in the presence of oxygen and water. This observation confirms fully the data collected by Gilchrist in *Chilota* and shows that the disappearance of luminescence points to the complete oxidation of the luminous material.

If the ground animals be mixed with water to which some sodium hyposulphite has been added to remove the oxygen, and a few drops of methylene blue which serves as an indicator for the presence of oxygen, the light which appears on addition of ether is visible after a longer time, and is brighter than in the control tube without the sodium hyposulphite. The flash of light is observed at the moment when on shaking, the oxygen dissolves in sufficient quantity so as to change the colorless methylene blue to the colored compound. This is apparently due to the nonoxidation of some of the granules, which were not completely destroyed by the action of enzymes or perhaps to the lessened activity of these enzymes due to the modified composition of the solution.

The different tissues of *Microscolex* contain a sufficient quantity of oxygen to allow luminescence as proved by vital staining with methylene blue. Nevertheless the light is not produced continually as in luminous bacteria, which fact must be taken as contradictory to the bacterial origin of luminescence in this animal.

It is well known that luminous bacteria cease to glow at a lower temperature than the secretions of animals which have luminescence of their own. Furthermore one of the constant characteristics of the bacterial light is the reappearance of the luminescence after cooling and of the same intensity as before heating. The living *Microscolex* kept in fresh water does not give off any light upon addition of ether or alcohol if previously heated to 60° C. The same was noticed if the dried material or the filter paper impregnated with luminous slime were tested for luminescence by pouring on them water heated to different temperatures from 55°–60° C. It may be mentioned here that after cooling the luminescence did not return in any case. These observations agree with the results obtained in other luminous animals whose self-luminescence is beyond any doubt, and differ widely from the behavior of luminous bacteria. Different results were obtained, however, with solutions prepared from the whole worms. In this case the maximum of temperature at which the light was observed reaches but 40° C. This difference may be explained by the quicker destruction of the luminous granules by the substances (enzymes) liberated from the rest of the body. Should this explanation prove true one cannot expect to find in the solution at a higher temperature unaltered luminous material which may react with the added ether.

In the older literature one finds statements that the dried luminous slime of the earthworms can be made luminous again by application of water. The same was found in my observations and this experiment can be repeated many times until complete oxidation of the luminous material. If the whole animals be dried in an evacuated desiccator the ground powder behaves like the fresh discharged slime, the intensity of luminescence being about the same. Even after three months the dry powder glows brilliantly if in water, the luminescence lasting for some time relatively to the amount of the powder. The dried bacteria however can be made luminous by moisture but only for a shorter time after desiccation, and the light is much fainter than that of the living microörganisms.

Pierantoni states that the addition of sodium chloride to the water in which the living specimens of *Microscolex phosphoreus* are kept inhibits their luminescence. In spite of many trials I have not been able to demonstrate the effect of different salt solutions when added to the powder from desiccated earthworms. The observations on *Microscolex* agree therefore with those of E. N. Harvey on *Cypridina* where the effect of different ions was not to be found. Pierantoni in his paper distinguishes two types of luminescence in *Microscolex*. Besides the external secretion of luminous slime he describes a general luminescence of the whole body, first visible at the posterior and anterior part, which takes place within the different tissues. This internal luminescence may be produced by the strong stimulating action of ammonia or alcohol. It seems to me that in many cases the ejaculated slime, through the movements of the animal, spreads over the whole body and sticks to the skin, thus imitating an internal luminescence. By rubbing the worm over the filter paper or cloth all the slime can be removed but in water, however, the viscous slime does not dissolve easily. In ammonia of the proper strength the motionless animal begins to glow, the posterior part glowing first. After some time the luminosity spreads over the whole body even if the animal be apparently dying. The same effect can be obtained with alcohol solution. With ether or chloroform I noticed only the external luminescence, the movements of the worm being for a longer time very energetic. Ammonia arrests almost immediately the muscular action. It is therefore to be understood that only in a few cases the ejaculation of the slime on application of ammonia was observed. The luminosity of the dying animal may be compared with the steady death glow as noticed in *Noctiluca* (E. B. Harvey, '17) and other forms. This internal luminescence may be hastened if the worm be rubbed gently with the finger when in ammonia or alcohol. If the animals are in a very poor condition they can also be made luminous by rubbing them, even without ammonia or alcohol. Sometimes the worms already luminescent can, if slightly pressed, throw the luminous slime through the anal or oral aperture. After the ejaculation of slime the animal loses its luminosity, which indicates that the internal luminescence cannot be taken as a different type from the external one. Pierantoni's view, which explains the internal luminosity as the continual glowing of the symbiontes inside the cells of the different tissues of the body, seems to me, therefore, a very improbable hypothesis.

DISCUSSION.

Summing up all the characteristics of the luminescence of *Microscolex phosphoreus* (the rôle of the nervous system, the effect of ether, temperature, the luminosity of desiccated worms) we can see that they do not agree with the observations on the properties of bacterial light but point to a luminescence of its own in this species of *Oligochæta*. It is very improbable that the luminous symbiontes may change their type of luminescence so completely as to behave like such animals as *Cypridina*, *Pholas*, *Noctiluca* and others, especially as the luminous bacteria living in luminous organs in some fishes and Cephalopods do not show such differences, though their shape may be modified. *Microscolex* and *Chilota* are therefore at least two earthworm species which are certainly not infected with luminous bacteria. *Allolobophora fœtida* (Vejdowsky) however may be occasionally infected by microörganisms or fungi, as only a few luminous specimens were found.

As I have mentioned before, the luminous material in *Microscolex* consists of small granules which usually begin to glow after the slime has been discharged. If the animal be dying however, they become luminous within the body cavity. It may be thought that the luminescence of the granules depends on an amount of oxygen which is not present in sufficient quantity in cœlomic fluid. If the worm be dying the amount of oxygen may be increased, but the experiments carried out by E. N. Harvey did not show any difference in the rate of permeability for oxygen between living and dead cells. One may suppose however that the luminescence begins when the cells disintegrate, which is corroborated by the fact that on rubbing the brightness of the slime increases. The process of breaking up the cells with granules within was observed by me several times after the slime was discharged. On one occasion Gilchrist noticed that the light was given off from the glowing particles situated in cells, which were in process of breaking up. I do not think that the granules can be luminous only during the time when they begin to be thrown off from the cells, for the centrifuged extract containing the free granules alone gives off light if ether be added, but it may be that they become luminous after they are liberated. It is possible that if the worm be dying the cells break up inside the body cavity. This process never occurs if the animal is in good condition. The accelerating effect of the rubbing may be explained by the increase of the process of breaking up the cells. In favor of such a view it may be noticed that the ejaculated slime which glows brightly always contains free granules in abundance, and their amount increases with time when the cells dissolve. It was impossible to prove this directly as the light of the granules is not strong enough to be seen under the microscope.

I wish to express my sincerest thanks to Professor E. N. Harvey for many suggestions and criticism. My thanks are also due to Professor R. Dohrn, Director of the Zoölogical Station in Naples and his staff for many courtesies shown me during my stay there.

Summary.

Microscolex phosphoreus is characterized by an external luminescence (except the steady death glow) which begins upon stimulation. All the properties of its light seem to show that this species has a luminescence of its own. The luminous material is represented by small granules situated in the protoplasm of the cells, which take their origin from the body cavity. The luminescence begins probably after the granules are liberated from the cells.

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CHROMOSOMAL VESICLES AND THE STRUCTURE OF THE RESTING NUCLEUS IN *PHASEOLUS.*

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In 1885 Rabl formulated a theory which is essentially our modern theory of continuity of chromosomes. Since that time cytologists and geneticists have very thoroughly discussed this problem, with its bearing on our conception of the structure of the nucleus and the related problems of genetics. Unfortunately, even at the present time, a large part of the evidence for the individuality of chromosomes is purely inferential; consequently the subject still rests on a rather hypothetical basis. Only in one case have the chromosomal vesicles with clear outlines been traced through the resting nucleus and in this instance the conditions were such that the observations cannot be applied to other forms with any degree of safety (Richards, 1917). Other workers have identified chromophilic bands in the resting nucleus as chromosomes, but the outlines of the vesicles were not visible (Sharp, 1914; Wenrich, 1916). In addition to the above isolated cases a great deal of strong inferential evidence for the individuality of chromosomes has been accumulated (Boveri, Conklin, etc.).

While engaged in studying the cytological changes accompanying the ripening and germination of the seeds of the common bean, *Phaseolus vulgaris*, a number of observations were made which indicated that *Phaseolus* affords favorable material for studying the reconstruction of daughter nuclei and interpreting their resting structure. This material was even more promising because of the fact that the resting nucleus of *Phaseolus* is quite similar to that of some other plants.

I wish to express my gratitude to Mr. G. T. Kline, artist, St. Louis University School of Medicine, for his skillful preparation of the plates, and to Professor Albert Kuntz for kindly reading the manuscript.

Material and Methods.

The root tips of growing *Phaseolus* seedlings were used. Since a matter of considerable controversy was the subject of this work it was thought desirable to use a number of fixatives.

Flemming's strong gave excellent results, though only the peripheral portion of the root could be used. Root tips treated with this fixative show three degrees of fixation. In the outer layers of cells the cytoplasm and nucleus appear quite normal, though the linin of the nucleus is not quite so accentuated as in Bouin's. The next few layers of cells appear to be poorly fixed, but are valuable for comparison with the outer cells. The innermost part of the root tip does not seem to have been penetrated at all.

Bouin's proved to be very valuable since it slightly swells the chromatin and accentuates the linin. There was no indication that the actual structure was hidden or changed, but rather that certain features of normal structure were made more evident. The cytoplasm was not as well fixed as in Flemming's strong. The tissue was fixed all the way through.

Alcoholic Bouin's and a mixture of three parts Bouin's to one part one per cent. osmic acid gave only fair results, inferior in all respects to Flemming's and Bouin's. Zenker's, Carnoy's and corrosive-acetic-alcohol shrunk the cells to such an extent that they were of no value whatever.

Very slow dehydration was found to be essential. Starting with thirty per cent. alcohol two to three days were consumed in the process of dehydration. The tips were embedded in hard paraffin and cut at 4 micra.

The sections were stained in iron-alum-hæmatoxylin and lightly counterstained with eosin.

Observations.

Since the principal object of this work is the interpretation of the structure of the resting nucleus the logical starting point is the reconstruction of daughter nuclei. This will be followed by a description of the resting nucleus and the account will end with the prophase.

Reconstruction of Daughter Nuclei.—One of the features probably responsible for the value of *Phaseolus* for a study of this subject is the uniformly small size of the short rod-like chromosomes. As is usually the case with chromosomes of this kind they migrate to the poles with their long axis parallel to the long axis of the spindle and the advancing chromosomes are so evenly placed on the spindle that an almost perfect ring is formed. This perfect order is maintained after the daughter chromosomes come in contact with each other at the poles and at the time when the apparent fusion occurs the chromosomal mass appears as a rectangle with its greatest dimension at right angles to the long axis of the spindle. At this time the outlines of the chromosomes are not clear and we can recognize the individual chromosomes only by the corrugation on the sides of the rectangle. The rounded ends of the chromosomes prevent their tips from coming in contact with each other and are responsible for the corrugations on the margins of the mass of chromosomes (Fig. 1). Shortly after the appearance of globules of achromatic material in the chromosomes the notched edges of the chromatin mass disappear and the outline of the reconstructing nucleus becomes smooth.

In rare instances the chromosomes are not perfectly parallel when they become contiguous with each other at the poles. In such cases the chromatin mass is not as regular as described above.

Figures 2 and 3 make a very important point for this description. Fig. 2 illustrates the beginning of vacuolization in one of the daughter nuclei, and in the other the vacuolization has proceeded to such an extent that distinctly clear areas are appearing in the proximal[1] ends of the chromosomes.

This figure also shows that the achromatic globules occur between the indentation on the proximal surface of the nucleus and that a linin strand runs inward from the indentation and joins that part of the chromatin mass which has not yet taken up achromatic material. A little later in the process of reconstruction the proximal side of the nucleus becomes less chromatic, the vacuolized portion of the chromosomes being more extensive. The indentations, especially on the proximal surface, are still quite pronounced and linin strands extending inward from them are also prominent.

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[1] Throughout this paper the ends of the chromosomes towards the equator of the spindle will be termed "proximal," while the opposite end will be called "distal."

It is well to emphasize at this point an interpretation of the linin strands which, doubtless has already occurred to the reader. The fact that they invariably run inward from the indentations on the surface of the chromatin mass which represent the points of contiguity of adjacent chromosomes strongly indicates that they as well as the superficial indentations, represent the line of contiguity of adjacent chromosomes. This also supports the conception of chromosome structure held by many zoölogists, notably Conklin, that the chromosome consists of a linin bag filled with chromatin. Achromatic globules appear in the chromatin groundwork of the interior of the chromosomes while the linin bag remains unaltered, except for the swelling due to increase of material within it. At this early stage of reconstruction there is no evidence whatever for the existence of linin strands within the external chromosomal sheath (Fig. 3).

The process of vacuolization normally continues until about three fourths of the length of the chromosome contains a large part of achromatic material, the so-called granules of chromatin occurring principally along the linin strands. The distal fourth of the chromosome is still a solid mass of chromatin and so it remains until the reconstruction of the nucleus is complete (Fig. 4). The imbibition of achromatin by the proximal ends of the chromosomes has not yet been completed. This continues and causes the increase in size of the daughter nucleus up to the time when it ceases swelling and enters the interphase or the resting period.

Since the distal fourth of the chromatin mass does not take up achromatin, it does not increase in size in harmony with the proximal portion of the chromosomes, and the latter tends to swell out over it and, as it were, to engulf it. This causes the linin strands, representing lines of contact of chromosomes, to change their position, and, ceasing to be parallel to the long axis of the cell, they become radiating strands with the non-vacuolized chromatin mass as their center. At the same time the solid distal ends of the chromosomes tend to clump. Fig. 4 illustrates a cell in which the daughter nuclei still have the non-vacuolized portion of every chromosome distinctly separated. From this point on they start joining so that nuclei may be found with only four, three or two masses of chromatin within them (Figs. 5 and 6). Fig. 6 illustrates two of these bodies in the act of joining.

As pointed out above the vacuolization of the proximal portion of the chromatin mass tends to turn the outer ends of the linin strands outward from the long axis of the cell, while the clumping of the solid ends of the chromosomes drags their other end towards a somewhat central point in the nucleus, resulting in the strands radiating from the chromatin mass, which might now be termed the nucleolus or karyosome, like the spokes of a wheel from the hub. The above gives only a two dimensional view of the process which, naturally, is not complete. The chromosomes migrate to the poles of the spindle covering it all the way around, consequently, when the proximal ends of the chromosomes swell and the distal ends join to form the nucleolus the linin strands radiate from the nucleolus, not like spokes from a hub, but like spines from the body of a bur.

When all of the small masses of chromatin first join to form the nucleolus the latter body is irregularly shaped, but it rapidly assumes the spherical form typical of nucleoli of resting nuclei. Fig. 8 represents a dividing cell in which the nucleolus in one of the daughter nuclei is almost spherical while that in the other daughter nucleus is irregular.

Interphase and Resting Nucleus.—The term "interphase" is used to signify the interkinetic period in the rapidly dividing cells of the root tip while the nuclei farther up the root that are dividing slowly or not at all will be called "resting" nuclei. The interphase nucleus exhibits a relatively large nucleolus which may contain one or more achromatic globules, and relatively little scattered chromatin. The linin strands which, as shown above, represent the line of contiguity of adjacent chromosomes, are quite evident in such nuclei. The few disperse chromatin granules are found principally around the periphery of the nucleus and even there are not sufficiently abundant to obscure the linin strands (Fig. 10). During the dormant period of the bean seed the scattered chromatin either enters the nucleolus or becomes closely appressed to the nuclear membrane, as has been previously shown (Kater, MSS.). In such nuclei there is nothing to obscure the linin strands, and, consequently, they are very generally visible (Fig. 12).

One might ask why the number of linin strands seen in the telophase and in resting nuclei is so small in comparison with the number of chromosomes in *Phaseolus*. It is only necessary to consider again that the nucleus has three dimensions and that, when observing a complete nucleus many of the linin strands are seen in end view, and it is only the ones in a plane parallel to the slide that are seen as definite lines.

In the resting nucleus there is a much greater abundance of scattered chromatin than in the interphase. Likewise there is a compensatory decrease in the size of the nucleolus and the scattered granules come up almost to the nucleolus, leaving a very small perinucleolar zone. When tissue is fixed in Bouin's small sections of the linin strands can be made out, but after fixation in Flemming's strong the linin strands are rarely seen in such nuclei. The abundance of scattered chromatin obscures them (Fig. 11). There can be no doubt as to their presence because in the same area of a ripe embryo, when the scattered chromatin has disappeared, they are quite evident and are invariably visible in interphase nuclei.

In properly fixed cells the nuclei have no resemblance whatever to the perfect reticulated network figured for plant nuclei by Gregoire (1904) and Sharp (1914). A somewhat similar structure is found in the second zone of Flemming's fixation, mentioned above. Slight indications of the chromatin bands which Sharp identifies as chromosomes may also be seen (Fig. 13). It seems probable that such slight distortion does not actually mislead the observer, but merely clumps the chromatin of each individual chromosome and enables the observer to trace structures which otherwise he could not recognize.

Before concluding this description of the resting nucleus we should emphasize the existing relationship between the chromosomes, as outlined in the description of the reconstruction of daughter nuclei. Every chromosome in the resting nucleus must be essentially a pentagon with four of the sides equal triangles while the fifth side has a convex surface and serves as a part of the nuclear membrane. The inner point of this wedge-like structure, where the four triangles come together, consists of non-vacuolated chromatin and makes up a part of the nucleolus. The outer part is principally achromatic and, consequently, much larger. Thus, every chromosome contributes to the formation of the nucleolus and likewise to the so-called nuclear reticulum. As stated above in distinguishing between interphase and rest, when the meristematic region moves forward leaving behind it cells that cease dividing we find an increase of scattered chromatin and a decrease in the size of the nucleolus. Since every chromosome enters into the formation of the nucleolus, this giving up of material by the latter body affects all of them and inasmuch as the nucleolus remains practically spherical the chromosomes apparently are equally active.

Prophase.—Before attempting to interpret the structure of the resting nucleus it is necessary that one study the development of that body in the telophase. It is equally necessary that one check these results by studying the behavior of the chromosomes during the prophase.

The onset of mitosis is first indicated by an increase of size and of the basophilic character of the scattered chromatin granules (Fig. 14). The next evident change is the appearance of considerable quantities of chromatin on the linin strands (Figs. 15). A little later the nucleus becomes smaller and consists almost entirely of chromatin. The linin strands are now covered with heavy bands of chromatin and the intervening spaces are well filled with large chromatin granules (Fig. 16).

The next change is important and introduces a very revolutionary fact. The nuclear membrane does not dissolve and disappear as has been universally described previously. Breaks appear in its wall, but each break leads into a short channel which terminates before reaching the general outline of the nucleolus, and this channel is bordered on either side by the end of a chromosome, now fully formed except that one end is still imbedded in the nucleolus. Fig. 17 shows a cell in which one side of the nuclear membrane is still intact and one can see that it appears to be continuous with the chromosomes projecting from the nucleolus on the other side.

The spherical outline of the nucleus entirely disappears in the manner described above and there remains in the place of the old nucleus a mass of chromosomes with one end of each still imbedded in the apparently homogeneous nucleolus and the other end projecting freely (Fig. 18). In order to return to the point at which this description started the chromosomes have only to separate. This is a gradual process. Cases may be observed in which only one or two chromosomes have become entirely separated from the rest of the mass (Fig. 19). From this condition we go, chromosome by chromosome, to the point where all or nearly all of them are separate (Fig. 20).

The chromosomes of *Phaseolus* are too small to be favorable for a study of the process of splitting and since that is not the subject of this work no careful attempt has been made to follow that phenomenon.

Historical and Discussion.

Reconstruction of Daughter Nuclei.—Phaseolus has been used by several investigators in studies of mitosis and nuclear structure. Wager (1904) has given a very excellent account of mitosis in this plant and, in view of the fact that he did not properly interpret his observations, the accuracy with which he figured them is remarkable. Although he did not follow the nuclear changes in the telophase closely enough to show conclusively that the linin strands, which apparently connect the nucleolus with the nuclear membrane in the resting stage, are the linin sheaths of chromosomes and that the chromosomes retain their individuality through the resting period, he, nevertheless, gives several very suggestive figures. Figs. 29 and 30 in his account are practically the same as Figs. 4 and 6 in the present work. The principal object of his study was to discover the origin of the nucleolus and his conclusion that it arises by the joining of non-vacuolated ends of chromosomes has been clearly verified by the present observations.

Wager observed and figured telophase conditions which, if they had been carried a little further, would have shown conclusively that the chromosomes of this plant are continuous from one mitosis to the next. He merits credit for being the first to make such clear observations of this phenomenon even though his interpretations were entirely beside the mark and his paper did not attract widespread attention.

This author figures the linin strands in resting nuclei as well as their development in the telophase. His observations on both of these phases have been violently attacked by Martins Mano (1905). Immediately after the appearance of Wager's article Martins Mano undertook to repeat his work on *Phaseolus*. The latter concluded that the nuclear reticulum arises by the "branching and anastamosing" of telophase chromosomes and that the nucleolus appears independently and rather suddenly after the reconstruction of the daughter nuclei is almost complete. His figures of the telophase, which led to the above incorrect interpretations, can be explained on the basis of Fig. 7. This figure illustrates a cell through which the plane of the section passed in such a way that a very small portion of one of the daughter nuclei appears in the section. The non-vacuolized portions of the chromosomes are entirely absent and, since only a small part of the side of the reconstituting nucleus is present the proximal ends of the chromosomal vesicles appear in cross section and consequently fail to show the even division of the nucleus by parallel linin strands. The other nucleus in this dividing cell exhibits the normal picture. Martins Mano admitted that structures similar to Wager's description were found, but claimed that the ones which he figures were of more frequent occurrence. In the light of the present work it can be safely said that Martins Mano must have exercised a great deal of patience in searching for the abnormal and infrequently occurring cells which he figured and with which he tried to support the theory that the reconstruction of nuclei is by the branching and anastamosing of telophase chromosomes, a theory which is incorrect in the case of *Phaseolus*. Martins Mano says that the linin strands which Wager figures as running from the nucleolus to the nuclear membrane do not exist, but are a part of the reticulum above the nucleolus. Their presence in the dormant seed, when the reticulum disappears shows that Wager was correct.

The great majority of described cases of chromosomal vesicles appearing during the reconstruction of nuclei are from animal eggs. Such structures have been described and discussed so frequently that it is not worth while to devote space to them here (Boveri, 1909; Moenkhaus, 1904; Conklin, 1912; Richards, 1917).

The vacuolization of plant chromosomes has not been so frequently described and is not so easily studied because the chromosomes do not take up as much achromatic material as do the chromosomes of animal eggs. The early vacuolization of the chromosomes of *Vicia* was studied by Sharp (1914). This plant has large chromosomes and affords very favorable material for the botanical approach to the subject. The chromosomes of *Phaseolus* are small and not suited to a study of this kind. Their great value for the present work lies in the fact that when the chromosomes come in contact with each other at the poles of the spindle they are perfectly parallel with each other. This fact, together with their rounded ends makes it possible to identify the linin strands as the linin sheaths of chromosomes.

Similar linin strands have been figured in the resting nuclei of other plants (Martins Mano, 1904; Bergs, 1906; Lutman, 1925). These investigators as well as Derschau (1904) have also described the telophase in a manner which indicates that the condition in those forms on which they were working is very similar to that of *Phaseolus*.

Prophase.—In the only case in which chromosomal vesicles have been traced through the resting stage of the nucleus (Richards, 1917) the figures are so clear and convincing that one has no cause whatever to doubt the endogenous formation of chromosomes. The new chromosomes are formed in the prophase vesicles and are set free by the dissolution of the old walls. After reading this account of the formation of chromosomes in *Fundulus* it was very surprising to find that they are not formed in the same way in *Phaseolus*, but that the chromosomal vesicles merely lose their achromatic content, contracting and giving rise to the new chromosomes, the old linin sheath apparently being continuous from one division through the next.

Wager concluded that the nuclear membrane of *Phaseolus* dissolves. However, his figures do not entirely justify that conclusion and indicate that his material was identical with that used in the present study. The author knows of no account at the present time which would justify the conclusion that in any other plants or animals the prophase chromosomes are formed in the same way as in the bean.

Martins Mano states that the nucleolus of *Phaseolus* does not contribute to the formation of chromosomes. The figures of the present paper as well as those of Wager are quite conclusive on this point and show that he was mistaken.

The Nucleolus or Karyosome.—In the many descriptions and discussions of chromatin nucleoli the various authors have not taken account of the fact that the nucleolus may be made up of a non-alveolized portion of every chromosome, as in the case in *Phaseolus.* If the chromatin nucleolus is a store-house for chromatin it is quite natural that it should be found at the meeting point of all the chromosomes, since the latter remain distinct throughout the resting phase of the nucleus.

Continuity of Chromosomes.—The significance of chromosomal continuity for various problems of cytology and genetics has been completely discussed previously, even before Richards had, for the first time, traced chromosomal vesicles through the interkinetic nucleus (Moenkhaus, 1904; Conklin, 1902; Wenrich, 1916; Richards, 1917; Wilson, 1925). A repetition of this discussion would be superfluous.

SUMMARY.

1. When the chromosomes of *Phaseolus* reach the poles of the spindle they come in contact with each other, all lying parallel to the long axis of the spindle. The individual chromosomes can be traced by the indentations on the surface of the chromatin mass.

2. Vacuolization of chromosomes begins in the end nearest the equator of the cell (the proximal end).

3. The distal ends of the chromosomes do not become alveolized, but join to form the chromatin nucleolus. There is no plasmasome present in the root-tip cells of *Phaseolus*.

4. The linin sheath of the chromosomes becomes visible in conjunction with the telophasic vacuolization and can be traced through the interphase and resting condition, thus showing that the chromosomes remain distinct from one mitosis to the next.

5. The chromosome is considered as a linin bag filled with chromatin. *Therefore, in the interkinetic nucleus the chromosomes are contiguous and the chromatin of adjacent chromosomes is discrete.*

6. The chromosomes arise in the prophase by the chromosomal vesicles losing the achromatic globules within them and contracting to form the apparently homogeneous metaphase chromosomes.

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EXPLANATION OF PLATES.

All figures drawn from sections 4 micra thick with aid of Abbe camera lucida and Leitz 2 mm. oil immersion lens. Figs. 1, 4, 7, 10, 11, 13, and 15 to 22 from material fixed in Flemming's strong; the remaining figures from sections fixed in Bouin's. All sections stained with iron-alum-hæmatoxylin and lightly counter-stained with eosin. With the exception of figures 21 and 22 all drawings represent structures in two dimensions. Magnification—1900 ×.

PLATE I.

FIG. 1. The chromosomes have become contiguous at the poles of the spindle. Individual chromosomes can be traced by their rounded ends.

FIG. 2. Vacuolization is beginning. Rounded ends still indicate limits of each chromosome.

FIG. 3. Vacuolization continued. Disperse chromatin granules in chromosomal vesicles.

FIG. 4. Note non-vacuolized portion of each chromosome. Linin strands represent line of contiguity of different chromosomes. Chromatin granules in vesicles.

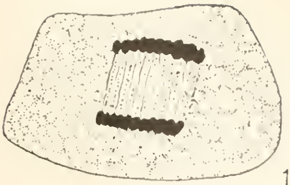
FIG. 5. The linin borders of the chromosomes are still prominent and the non-vacuolized ends of the chromosomes are joining.

FIG. 6. A continuation of the changes illustrated by Fig. 5. Note lower nucleus where the two last non-vacuolized portions of chromatin are joining to form chromatin nucleolus or karyosome. Disperse chromatin granules are located principally around the periphery of the nucleus.

FIG. 7. Approximately the same stage of development as Fig. 6. The section was so cut that the nucleolus does not appear in one of the daughter nuclei. Note that the linin strands are not parallel in this one.

FIG. 8. Completion of the joining of the non-vacuolized chromosome ends to form the nucleoli. Nucleolus approximately spherical in one daughter nucleus. Linin sheaths of chromosomes still prominent.

FIG. 9. Polar view of reconstructing nucleus. This nucleus is at about the same stage of development as Fig. 5. The linin strands are not parallel as in side view.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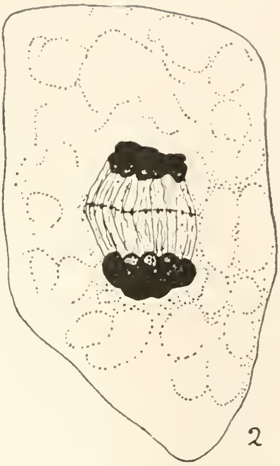
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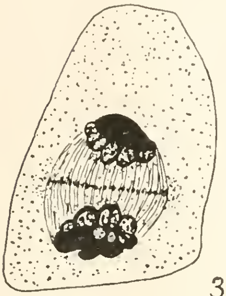
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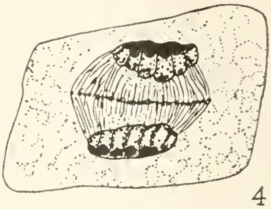
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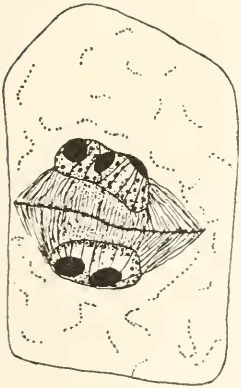
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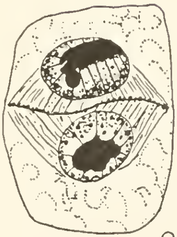
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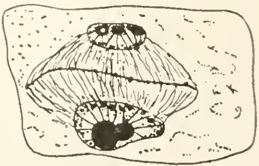
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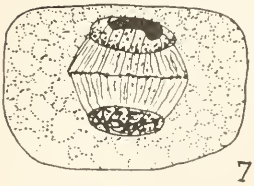
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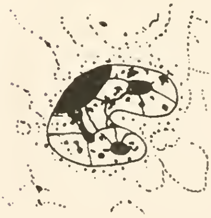
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PLATE II.

FIG. 10. Interphase nucleus from the meristematic tissue at the tip of the root. Disperse chromatin granules around the periphery of the nucleus. Linin sheaths of chromosomes prominent.

FIG. 11. Resting nucleus from slowly growing region of the root. Nucleolus smaller than in interphase and disperse chromatin so abundant that the linin strands are obscured.

FIG. 12. Cell from the radicle of a dry mature bean embryo. Disperse chromatin has disappeared. Linin sheaths of chromosomes are very prominent.

FIG. 13. Resting cell illustrating poor fixation by Flemming's solution, excepting a few peripheral layers of cells.

FIG. 14. Early prophase. The disperse chromatin granules are becoming larger, fewer, and stain more darkly.

FIG. 15. Prophase continued. The disperse granules are larger and a heavy band of chromatin is appearing along the linin strands.

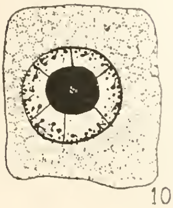
FIG. 16. A continuation of processes illustrated in Fig. 15.

FIG. 17. The chromosomal vesicles on one side of the nucleus have lost all achromatic material and are contracting to form chromosomes. Note that the nuclear membrane of the other side is continuous with the nearest formed chromosomes.

FIGS. 18, 19 AND 20. Further steps in the contractions of chromosomal vesicles and separation of the chromosomes.

FIGS. 21 AND 22. Drawings showing the chromosome elements in three dimensions. Compare 21 to 17 and 22 to 19.

PLATE II.



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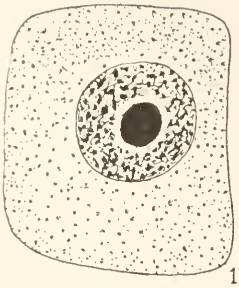

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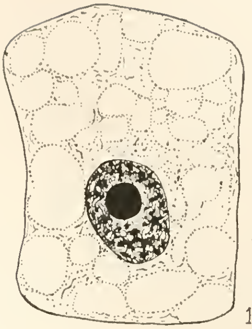

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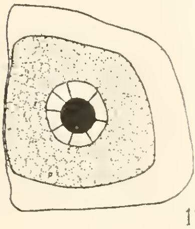

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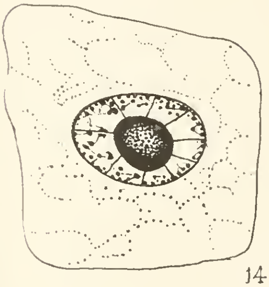

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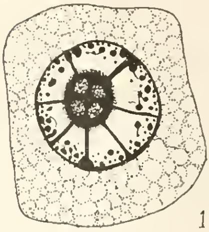

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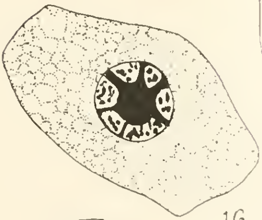

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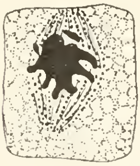

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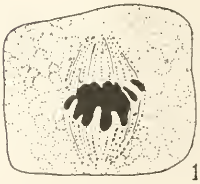

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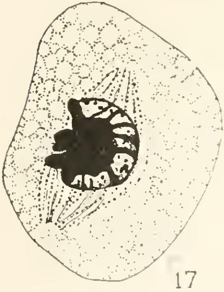

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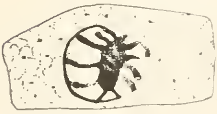

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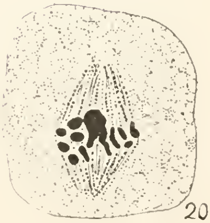

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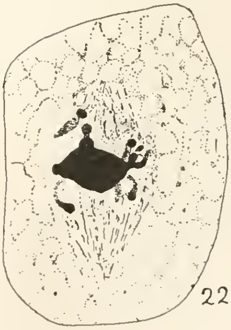

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BIOLOGICAL BULLETIN

GENETIC STUDIES IN POULTRY.[1]

I. THE SEX RATIO IN THE DOMESTIC FOWL.

W. V. LAMBERT AND C. W. KNOX.

INTRODUCTION.

In comparison with mammals the sex ratio in birds has been studied by relatively few investigators. The first report on the sex ratio in birds appears to have been made by Darwin in 1871. He noted that the per cent. of males to females in 1,001 chickens raised by Stretch was 48.65. Field (1901) in 2,105 chickens reported a sex ratio of 44.63, Thomsen (1911) in 805 observations a sex ratio of 47.82, Pearl (1917) in 22,791 cases a percentage of 48.57 males, Crew and Huxley (1923) a sex ratio of 49.26 in a total of 753 chicks and embryos examined, Jull (1924) a sex ratio of 48.88 on 2,396 individuals sexed, and Mussehl (1924) a percentage of 52.24 males from a total of 1,514 chicks upon which observations were made. In pigeons Cole and Kirkpatrick (1915) found that the per cent. of males to females was 51.06 on a total of 1,800 squabs and embryos examined.

The above data represent the total ratios observed by the various investigators on living chicks and dead embryos. With respect to the prenatal sex ratio, however, the data are less extensive, although the sex of the chicken may be quite readily told macroscopically by the ninth day of incubation. Of those reporting on the prenatal sex ratio, Pearl (1917) has the most extensive data, and he gives a sex ratio of 48.3 from a total of 1,921 embryos dying between the tenth day of incubation and hatching. Jull (1924) found a sex ratio of 47.18 in embryos dying after the eleventh day of incubation taken as an average of the results of continuous hatches throughout a three-year period. Thomsen (1911) in a total of 805 embryos examined before hatching found a ratio of 47.82, while Crew and Huxley (1923) observed a prenatal sex ratio of 45.24 out of a total of 420 embryos examined. In the latter case, some of the parents of the embryos were subjected to treatment with a thyroid extract.

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Material and Methods.

The material for the present study was obtained primarily from the F_1 and F_2 generations and backcross chicks of crosses made between the Rhode Island Red and White Leghorn breeds, and also of F_1 data from crosses made reciprocally between the Black Langshan and White Plymouth Rock, the Black Langshan and Buff Orpington, and the White Plymouth Rock and Buff Orpington. In addition a number of observations were made on data from the White Leghorn breed.

All eggs were candled on the sixth, twelfth, and eighteenth days of incubation, and embryos found dead on these respective days were classified as D_1, D_2 and D_3. All embryos found dead between the eighteenth day of incubation and the day of hatching were listed as dead in the shell (*DS*). All chicks of the D_3 and *DS* classes, and chicks dying before it was possible to determine their sex from the external appearance, were dissected and the sex determined in that manner. The sex ratio on all living chicks was determined as soon as that was possible from their external appearance.

In 1925 the total sex ratio from 121 hens between the hatching dates of March 4 and May 6 was obtained with the exception of losses due to straying, loss of bands, predatory enemies, and losses due to fire which destroyed a portion of the hatch of one week. Data are given showing the number of individuals upon which the sex was not determined, but there is no reason to believe that the sex ratio of these chicks would have deviated greatly from the ratios observed.

In addition to the above, observations were made upon the live chicks and dead embryos of a number of hens during the years

1924 and 1925. These data are not continuous throughout the hatching season but represent only data secured at various intervals during the hatching season. The record on any one hen, however, is complete for the period in which the observations were made.

The sex ratio as used herein expresses the percentage of males to females in the population.

RESULTS.

During the two years a grand total of 2,910 embryos and living chickens was examined. Of these 1,488 were males and 1,422 females or a sex ratio of 51.13. The results of the two years records are shown in Table I.

TABLE I.

THE TOTAL NUMBER OF MALES AND FEMALES OBSERVED AND THE SEX RATIO FOUND IN THE CHICKS HATCHING AND IN THE DEAD EMBRYOS.

Year.	D_3.			*DS*.			Chicks Hatching.			Totals.		
	♂♂	♀♀	R♂♂	♂♂	♀♀	R♂♂	♂♂	♀♀	R♂♂	♂♂	♀♀	R♂♂
1924......	13	8	61.90	26	34	43.33	68	81	45.64	107	123	46.52
1925......	126	102	55.26	374	365	50.61	881	832	51.43	1,381	1,299	51.53
Totals....	139	110	55.82	400	399	50.06	949	913	50.97	1,488	1,422	51.13

It will be noted here that the sex ratio in each class, namely D_3, *DS* and living chicks, is over fifty, it being 55.82 in the D_3, 50.06 in the *DS* and 50.97 in the living chicks. The results in each of the two years are not in very close agreement although the same general trend exists in each case.

From the 2,910 individuals shown in Table I. a complete sex ratio was secured on 2,266 chicks and embryos developing from the eggs produced by 121 hens during the season of 1925. Of all eggs used from this group of 121 hens and that developed beyond the twelfth day of incubation, barring those chicks lost due to the causes previously mentioned, a complete sex ratio was obtained between the hatching dates of March 4 and May 6. These data are shown in Table II.

TABLE II.

THE COMPLETE SEX RATIO FOR DEAD EMBRYOS AND CHICKS HATCHING DURING SUCCESSIVE TWO-WEEK INTERVALS FOR 121 HENS DURING THE NORMAL HATCHING SEASON OF 1925.

Hatching Dates.	*D*3.			*DS.*			Chicks Hatching.			Totals.		
	♂♂	♀♀	R♂♂	♂♂	♀♀	R♂♂	♂♂	♀♀	R♂♂	♂♂	♀♀	R♂♂
March 4 and 11	11	6	64.70	71	51	58.20	134	155	46.37	216	212	50.47
March 18 and 25	28	25	52.83	66	78	44.44	113	123	47.88	207	226	47.81
April 1 and 8	45	36	55.55	86	58	59.72	243	207	54.00	374	301	55.41
April 15 and 22	15	9	62.50	29	32	47.54	176	174	50.28	220	215	50.57
April 29 and May 6	3	6	33.33	12	18	40.00	138	118	53.91	153	142	51.86
Totals	102	82	55.43	264	237	52.69	804	777	50.85	1,170	1,096	51.63
Per cents	4.50	3.62		11.65	10.46		35.48	34.29		51.63	48.37	

Of the 2,266 individuals shown in the above table 8.12 per cent. were embryos dead between the twelfth and eighteenth days of incubation, 22.11 per cent. between the eighteenth and twenty-first days, and 69.77 per cent. were chicks sexed after hatching. It is interesting to note in this connection that the most critical period of development is apparently between the eighteenth and twenty-first days of incubation. Of all eggs set and dying in the embryonic stage, 361 or 34.5 per cent. died before the twelfth day of incubation, 184 or 17.6 per cent. between the twelfth and eighteenth days, and 501 or 47.9 per cent. during the last three days of incubation.

The sex ratio is listed each week for the three classes D_3, *DS* and chicks hatched respectively, together with the totals for each week and the total ratio of each class for the entire period with the corresponding ratio of males. These data in Table II. represent a total of 79.20 per cent. of all fertile eggs set during this time. Of the remaining 20.8 per cent. upon which the sex was not determined 12.62 per cent. or 361 died before the twelfth day of incubation and 8.18 per cent. or 234 were lost due to the causes listed.

In the D_3 class with the exception of the last two weeks interval the sex ratio is comparatively high, ranging from 52.83 to 64.70. However, no general trend is apparent during the consecutive intervals as the highest sex ratio appears in the interval from March 4 to 11 and the next highest in the period of April 15 to 22.

Of those embryos dying between the eighteenth and twenty-first days of incubation no general trend of the sex ratio is likewise apparent. In general, however, the sex ratio is lower than among the D_3 class.

Of the chicks sexed after hatching no particular trend is noted in the sex ratio from the beginning to the end of the hatching season. The per cent. of males of the chicks hatching varies from 46.37 to 54.00 with an average of 50.85. From the total of 2,266 chicks and embryos examined 1,170 or 51.63 per cent. are males and 1,096 or 48.37 per cent. females.

Some of the data included in Table I. were not complete for the whole season. These were obtained in both 1924 and 1925 and were tabulated without regard to the week in which they were obtained. On a few of the hens only data from the dead embryos were included, whereas, in others both data from dead embryos and chicks were included. In all cases, however, where the sex ratio of chicks was determined it represented a total hatch of those particular hens for that period. In other words it represents the complete record of the hens for any one interval. These unclassified data appear in Table III.

Table III.

The Observed Sex Ratio in Some Unclassified Data Obtained During the Normal Hatching Seasons of 1924 and 1925.

Year.	D_3.			*DS.*			Chicks Hatching.			Total.		
	♂♂	♀♀	R♂♂	♂♂	♀♀	R♂♂	♂♂	♀♀	R♂♂	♂♂	♀♀	R♂♂
1924.	24	20	54.55	110	128	46.22	77	55	58.23	211	203	50.97
1925.	13	8	61.90	26	34	43.33	68	81	45.64	107	123	46.52
Totals.	37	28	56.92	136	162	45.64	145	136	51.60	318	326	49.38

The large number of chicks dead between the eighteenth and twenty-first days of incubation appearing in the 1924 data is due to the fact, as mentioned before, that in some cases only the dead embryos of certain hens were sexed.

In this as in the preceding tables no very definite trend to the sex ratio is apparent. While there seems to be an excess of males in the D_3 column and an excess of females in the *DS* column the numbers are probably not large enough to indicate a selective mortality at the different stages of incubation. This would appear especially true since no similar condition appears for the more extensive data shown in Table II. In the chicks hatching listed in Table III. a high sex ratio occurred in 1924 and a low sex ratio in 1925. Hence it would appear that the variations noted in this table are probably due to the small numbers.

From the methodological standpoint Pearl (1917) has indicated the advantage of basing the sex ratio on families of ten or more individuals. He has compared the mean sex ratio of families of various sizes and finds the following ratios in each:

	R♂♂
Families of 10 and over	48.57 ± 0.28
Families of 4–9 inclusive	49.39 ± 0.84
Families of 1–3 inclusive	55.07 ± 2.11
Families of 4 and over	48.80 ± 0.33
Families all sizes	49.45

It becomes apparent from these figures that the smaller families are more likely to show extreme values of the sex ratio. In fact, according to Pearl, ratios above 80 and under 20 occur very rarely in families of ten or more.

The ratio of males for different colonies in the present experiment together with the number of hens in each colony is shown in Table IV.

Table IV.

The Sex Ratio for the Data Shown in Table II. Listed According to Breeding and Colonies.

Breeding.	No. of Birds in Colony.	D_3.		*DS*.		Chicks Hatching.		Total.		R♂♂
		♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	
F_2 (White Leghorn by Rhode Island Red)	17	11	9	44	56	188	202	243	269	47.46
F_1 (Black Langshan ♂, Buff Orpington ♀♀, White Plymouth Rock ♀♀)	10	9	7	26	24	41	42	77	73	51.33
White Leghorn	13	14	11	19	21	73	52	106	84	55.79
F_1 (Buff Orpington ♂, White Plymouth Rock ♀♀, Black Langshan ♀♀)	9	13	3	8	8	62	69	85	82	50.90
F_1 (White Plymouth Rock ♂, Buff Orpington ♀♀, Black Langshan ♀♀)	7	2	4	17	9	30	26	49	39	55.68
F_1 ♀♀ (White Leghorn ♂)	12	8	10	31	21	83	74	122	106	53.51
F_1 ♀♀ (Rhode Island Red ♂)	11	16	11	17	24	79	76	112	111	50.22
F_1 ♂ (Rhode Island Red ♀♀)	11	8	6	24	20	54	51	86	78	52.44
F_1 ♂ (White Leghorn ♀♀)	11	19	12	12	14	43	49	74	76	49.33
White Leghorn	5	0	1	4	6	33	36	38	43	46.91
White Leghorn	15	2	8	62	34	114	91	178	135	56.87

The range in the data is from 46.91 in the smallest colony with a total of five birds to 56.87 per cent. males in a colony with fifteen birds. The average sex ratio for all colonies is 51.63.

Both the high and low sex ratios appear in colonies of White Leghorns. The individual range in the colony showing the lowest per cent. of males was from 33.3 to 66.7 per cent. In the colony showing the highest sex ratio the range was from 44.4 to 85.7 per cent. In tabulating this range only birds producing ten or more chicks and embryos that were sexed were included. This included all individuals in the small colony and fourteen out of the fifteen in the large colony. The average sex ratio in the last case when determined for the fourteen birds having 10 or more sexed offspring was 59.2. It was thought necessary in determining the range to include only birds having ten or more sexed offspring as any smaller number than that would hardly give a representative sample.

From the total of eleven colonies only three show sex ratios of less than 50. No relationship appears to exist between breeds and the sex ratio for the hybrids show about the same variation as do the pure breeds.

The Influence of Egg Weight and Production upon the Sex Ratio.

Jull (1924) found no relationship between average weight of eggs and the sex ratio, and Jull and Quinn (1925) found no correlation between the weights of individual eggs and the chicks hatching from them.

Since the average egg weights for all F_1 hens used in this experiment were available as well as the sex ratio for these hens, a correlation between these two variables has been calculated. In this study only those hens that have a total of ten or more chicks or embryos upon which the sex had been determined were included, the total number being thirty-nine. The correlation coefficient for egg weight and percentage of males was -0.060 ± 0.108. This correlation being smaller than its probable error certainly indicates that no direct relationship exists between these variables.

On this same group of F_1 birds the rate of production preceding the hatching season has not influenced the sex ratio. In this calculation the percentage rate of production was used. This rate of production was used because the birds began laying at

TABLE V.

THE CORRELATION BETWEEN THE AVERAGE INDIVIDUAL EGG WEIGHTS, THE ACTUAL EGG PRODUCTION DURING THE HATCHING SEASON, AND THE RATE OF ANTECEDENT PRODUCTION RESPECTIVELY, WITH THE SEX RATIO AS THE DEPENDENT VARIABLE.

Variables.	Range.	Mean.	Coefficient of Variability.	Standard Deviation.	Correlation Coefficient.
A. Average individual egg weights (grams)	46.9–60.3	53.07 ±0.32	7.51 ±0.57	2.93 ±0.22	*A–D* −0.060 ±0.108
B. Actual egg production during hatching season	22–47	36.50 ±0.65	15.51 ±1.20	6.05 ±0.46	*B–D* −0.009 ±0.108
C. Antecedent egg production (per cent.)	11.1–57.9	43.00 ±1.16	28.35 ±2.22	10.49 ±0.82	*C–D* −0.048 ±0.111
D. Sex ratio (per cent.)	37.0–81.8	51.26 ±1.06	25.03 ±1.91	9.76 ±0.75	

different dates and the rate was determined by dividing the total number of eggs laid by the number of days between the date of first egg and the beginning of the hatching season. The correlation cœfficient in this case was − 0.048 ± 0.111. This correlation likewise is not significant and indicates, in this particular group of birds, that preceding production has not influenced the sex ratio.

In a study of the sex ratio based on hatches throughout the entire year Jull (1924) found a correlation of − 0.704 ± 0.031 between the sex ratio and antecedent egg production. As the season advances the ratio of males decreases, and Jull concludes that the cause for this decrease is directly related to the antecedent egg production.

The rate of egg production during the hatching season, likewise, was found not to have influenced the sex ratio in this group of birds. The correlation cœfficient between total production during the hatching season and the sex ratio was − 0.009 ± 0.108. This correlation, like the two preceding, is not significant being much smaller than its probable error.

Since the correlation cœfficients in all three cases were negative and smaller than their respective probable errors the conclusion is justified that neither mean egg weight, as measured for each bird nor the rate of production both preceding and during the hatching season has influenced the sex ratio. In other words there is no tendency for those birds laying the fastest or laying the heaviest eggs to produce more or less males than the birds producing the fewest or the lightest eggs.

The correlations between these three variables together with their respective ranges, means, cœfficients of variability, and standard deviations are shown in Table V.

Discussion.

The data presented herein for the most part show a higher sex ratio than has been reported by other investigators. Musehl (1924) reporting a sex ratio of 52.24 per cent. is the only one reporting a higher percentage of males. Cole and Kirkpatrick (1915) report approximately the same sex ratio in pigeons as is reported by the present writers, namely 51.06 per cent. All other studies have indicated a ratio of less than fifty per cent. males, both for living chicks and dead embryos. No reason for this higher sex ratio is apparent. It is true that a number of the birds were lost due to various causes but the writers have no reason to believe that there was a selective mortality of the chicks that were lost.

The proportion of males among the embryos dying between the twelfth day and eighteenth day of incubation is rather high, being 55.82. However, if reference is made to Table IV. it may be seen that it does not hold consistently true that the males exceed the females in the D_3 class. In the *DS* class there is practically an equality of males and females, the ratio of males being 50.06. All previous investigators reporting on the prenatal sex ratio alone in birds have reported a sex ratio less than fifty per cent. They likewise have seemed agreed that a selective mortality of one sex or the other previous to hatching does not occur.

The number of birds upon which the correlations have been calculated is small but the results found here are in general agreement with those of other investigators. More of the birds were not included in these studies because the mean egg weight varies for different breeds and in most cases the antecedent egg product on on the birds used in the various crosses was not known.

SUMMARY.

1. The sex ratio for 2,910 chicks and embryos examined was 51.13.

2. The sex ratio for total living chicks was 50.97, for embryos dying between the eighteenth and twenty-first days of incubation 50.06, and for embryos dying between the twelfth and eighteenth days 55.82 per cent.

3. No tendency for an increase or a decrease in the sex ratio as the hatching season progressed was noted.

4. The sex ratio of hybrid birds is approximately the same as for that of the pure breeds observed.

5. No relationship between the sex ratio and the factors of mean individual egg weight, antecedent egg production, and actual egg production during the hatching season is apparent.

The respective correlations found between these variables were -0.060 ± 0.108; -0.009 ± 0.108; and -0.048 ± 0.111.

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LIFE CYCLE OF *NASSULA ORNATA* AND *NASSULA ELEGANS:* ARE THESE SPECIES VALID?

EDNA McNALLY.

I. Description.

Nassula is a ciliated protozoan living in fresh water where there is an abundance of *Oscillatoria* and not too much sunlight.

Laboratory cultures of *Nassula* for these experiments were maintained by placing them in ordinary square watch glasses with spring water and fresh *Oscillatoria*, and these in turn, placed in large covered glass vessels containing a small quantity of water. The cultures were kept in subdued light as direct sunlight was found to be fatal. All work on *Nassula* was done with pure lines started from one individual and maintained for nearly a year.

The genus *Nassula* was first described by Eherenberg about 1830. His description includes the species *Nassula ornata* and *Nassula elegans*, the main difference between the two being color, shape, and size. *N. ornata* he describes as being ovate or cylindrical, dark green or violet in color and about 200–240 micra in length. *N. elegans*, he gives as varying in color from red to transparent, elongated, and about 160 micra in length (Eyferth, 1900).

However there is great confusion in the classification of *Nassula*. Pritchard (61), describes *N. ornata* and *N. viridis* as the same species while Eyferth (00), describes *N. ornata* and *N. aurea* as the same. The species *N. elegans* as described by Pritchard is exactly opposite to the description given by Cohn (53), for the same species.

Not only the species are confused, but also the genus, as is shown by the following: "Stein hints it as probable that this species (*N. aurea*) and *N. viridis*, *Chilodon aureas* and *Ch. ornatus* are merely different stages of the same animal" (Pritchard, 61).

The entire body of *Nassula* is covered with cilia appearing in rows, approximately longitudinally disposed, over its surface.

The nuclear complex is peculiar in that it is not differentiated into micro- and macro-nuclei but is very similar to the nucleus of *Amœba proteus*. This statement does not agree with Cohn (53), who says of *N. elegans*, "Nucleus elliptic, with nucleolus lodged at one end." And Pritchard (61), with *N. elegans* in mind, thinks the "nucleus is stoutly clavate, and terminated by a small oblong nucleolus at its narrower extremity." The nucleus does not occupy a fixed position but is moved around by the streaming of the protoplasm.

The mouth opens into a pharyngeal apparatus composed of about twenty-five pharyngeal rods arranged in the form of a truncated cone, with the base anteriorly directed. This pharyngeal apparatus is described by all of the above mentioned authors for all species with the exception of Cohn (53), who states that there is no such apparatus in *N. elegans*. About one third of the way posterior to the base of this basket-like cone appears to be a "band of refractive protoplasm," as described by Eherenberg (00), for *N. elegans*. Nothing was said of this ring in *N. ornata*. However, I have been able to demonstrate this ring in prepared material of both species. Eherenberg probably overlooked it in living specimens of *N. ornata* because of its being obscured by the food vacuoles. This mechanism seems to serve as a support to the pharyngeal rods and also aids in drawing *Oscillatoria* filaments through the pharynx into the body of the animal.

Food vacuoles are numerous and vary in number and color with the amount of food injested and the stage of digestion of this food. A freshly fed *Nassula* contains so many food vacuoles of such dark color that the other morphological details cannot be made out with certainty. Just after injestion of food the vacuoles are brown or dark green due to the color of the *Oscillatoria*, but as digestion proceeds they are changed to a shade of purple, then pink and finally faint straw color. All these stages may be encountered at one time in the same individual.

The contractile vacuole, of which there is but one, is peculiar. It is stationary and is the point toward which the streaming of the protoplasm is directed. As metabolism ensues minute vacuoles of clear fluid are formed throughout the protoplasm. With the streaming of the protoplasm these vacuoles are brought into close contact with the point at which the contractile vacuole is formed. As the minute vacuoles come together they fuse to form a larger vacuole, which, when its maximum size is reached, is discharged. Thus there can always be seen a larger contractile vacuole with smaller vacuoles of various sizes surrounding it, while others are drifting through the cytoplasm toward it. At the point of excretion is situated a wedge-shaped pore connecting the contractile vacuole with the outside of the animal, and through which the waste material passes. Waste material is discharged at the rate of about eighty seconds.

Nassula thrives only on *Oscillatoria*. Although I have observed *Nassula* containing a few desmids, I have never been able to maintain a culture using desmids as food. *Nassula* divides by transverse fission. The nuclear conditions during division have not yet been completely worked out but work is being done at the present time on this subject. Binary fission is found to occur more frequently in older cultures.

2. No Warrant for Two Species.

The amount of food present plays a great part in the life history of *Nassula*. As long as there is an abundance of *Oscillatoria* the animals, which were classified as *N. ornata* by Eherenberg, live and multiply in the normal way and continue to present the color, size and contour of *N. ornata*. But as the food supply becomes exhausted they undergo noticeable changes in these features. As stated above *N. ornata* is normally ovate or cylindrical, brown or dark green in color and about 200 micra long, but as the food supply diminishes the animal becomes very much smaller and is decidedly longer than it is wide. The color of the food vacuoles changes to a faint red. In this condition it meets all descriptions of *N. elegans*. If, when this stage is reached, *Oscillatoria* is fed to the animals, they immediately attach themselves to the filaments to feed. Several hours later they will be seen to contain numerous food vacuoles of very dark color and their normal size is again reached, now meeting Eherenberg's description of *N. ornata*. Thus, by withholding food, *N. ornata* may be made to assume the characteristics of *N. elegans*. Age of the culture also, as over against lack of food, plays a small part in the converting of *N. ornata* into the so-called *N. elegans*. Older cultures of *N. ornata* change a short while before those in which the water is fresher. This will be seen by referring to Table I. Hence we find that *N. ornata* and *N. elegans* are but metabolic phases of a single species based upon nutritional and toxic conditions.

3. ENCYSTMENT.

As stated above, after food has been withheld from *N. ornata* the animals develop the characteristics of *N. elegans*. If now, food is again added to these specimens a reversal will occur and the animals will again appear as *N. ornata*. This may be carried on indefinitely making alternate sequences of the forms so long as food is withheld and added at the proper time and in the proper sequences. However, if no food is added, the animals eventually become much lighter in color than even *N. elegans*, becoming almost transparent. Then encystment takes place. Thus it happens that they enter the cysts as *N. elegans*, *N. ornata* has not been observed to encyst.

Age of the culture aids in encystment but is not the basic cause as will be seen from the following specific experiment:

Four cultures were started as follows: All the *Nassulas* being taken from the same culture which was a sub-culture of the original pure line.

Culture No. 1.—Water from a culture of *Nassulas* several weeks old was filtered and placed in a watch glass with approximately twenty-five *N. ornata*. No *Oscillatoria* was present.

Culture No. 2.—Fresh spring water, about twenty-five *N. ornata* and no *Oscillatoria*.

Culture No. 3.—Water from the same vessel as No. 1, about twenty-five *N. ornata* and an abundance of *Oscillatoria*.

Culture No. 4.—Fresh spring water, about twenty-five *N. ornata*, and an abundance of *Oscillatoria*.

All of the four cultures were kept in square watch glasses and these placed in a larger glass dish so that the external conditions were the same.

The cysts are formed along the sides of the vessel or in the detritus on the bottom of the vessel. They are spheroidal in

TABLE I.

Date.	I.	II.	III.	IV.
May 5th.......	*N. ornata*	*N. ornata*	*N. ornata*	*N. ornata*
May 6th.......	*N. elegans*	*N. elegans*	*N. ornata*	*N. ornata*
May 7th.......	*N. elegans* (a few cysts)	*N. elegans* (no cysts)	*N. ornata* (no cysts)	*N. ornata* (no cysts)
May 8th.......	*N. elegans* (about ½ cysts)	*N. elegans* (a few cysts)	*N. ornata* (no cysts)	*N. ornata* (no cysts)
May 9th.......	All encysted	*N. elegans* (a few cysts)	*N. ornata* (no cysts)	*N. ornata* (no cysts)
May 10th......	All encysted	*N. elegans* (about ½ cysts)	*N. ornata* (no cysts)	*N. ornata* (no cysts)
May 11th......	All encysted	All encysted	*N. ornata* (no cysts)	*N. ornata* (no cysts)

shape, brown and the wall is rather thick, presenting a scalloped appearance. They are a great deal smaller than the normal animals and in a newly formed cyst a light spot, the nucleus can be seen.

Excystment can be brought about by the addition of fresh water and *Oscillatoria*. After excystment the animal has the characteristics of *N. elegans* until it encounters food. A few hours after feeding it again presents the characteristics of *N. ornata*.

4. CONJUGATION.

In old cultures low in food supply, in which because of their age and food scarcity, encystment might be expected, conjugation has been encountered. Conjugation has only been observed under such conditions and always occurring after the animals have undergone the change from *N. ornata* to *N. elegans*. Thus, these adverse conditions of the culture seem to play the greatest rôle in conjugation.

The animals approach each other and fuse end to end. The nuclear stages during conjugation have not yet been worked out, but observations of living specimens as well as prepared materials show that the nucleus is not broken down in the process. Calkins (26) states that in many of the protozoa which have been thought to possess a single nucleus, there are in reality both micro- and macro-nuclei. These two nuclei are so closely associated that the micro-nucleus is not visible until conjugation takes place. I am convinced that this is not the case in *Nassula*, for during conjugation of protozoa where there are both types of nuclei, the macro-nucleus breaks down and the micro-nucleus takes the active part. In *Nassula*, as stated above, there is no such process, the conspicuous nucleus, which according to Calkins statement would be the macro-nucleus, functioning as does a micro-nucleus during conjugation.

Many thanks are due Dr. W. A. Kepner of this Laboratory, for his valuable suggestions and aid in carrying on this work, and Miss Margaret Haase for the drawing.

SUMMARY.

1. *Nassula ornata* and *Nassula elegans* represent not two species, but two metabolic stages of a single species.

2. The well nourished phase, known as *Nassula ornata*, does not encyst. Lack of food, first, converts *Nassula ornata* into *Nassula elegans*, and, in time, drives the forms as *Nassula elegans* into encystment.

3. Binary fission occurs more frequently in the poorly fed forms (*Nassula elegans*) than in the well-nourished forms (*Nassula ornata*).

4. There is but one nucleus in this ciliate, there being no differentiation of the nuclear complex into micro- and macronuclei.

5. Conjugation involving this single nucleus takes place in stocks that have aged.

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'00 Einfachste Lebensformen.
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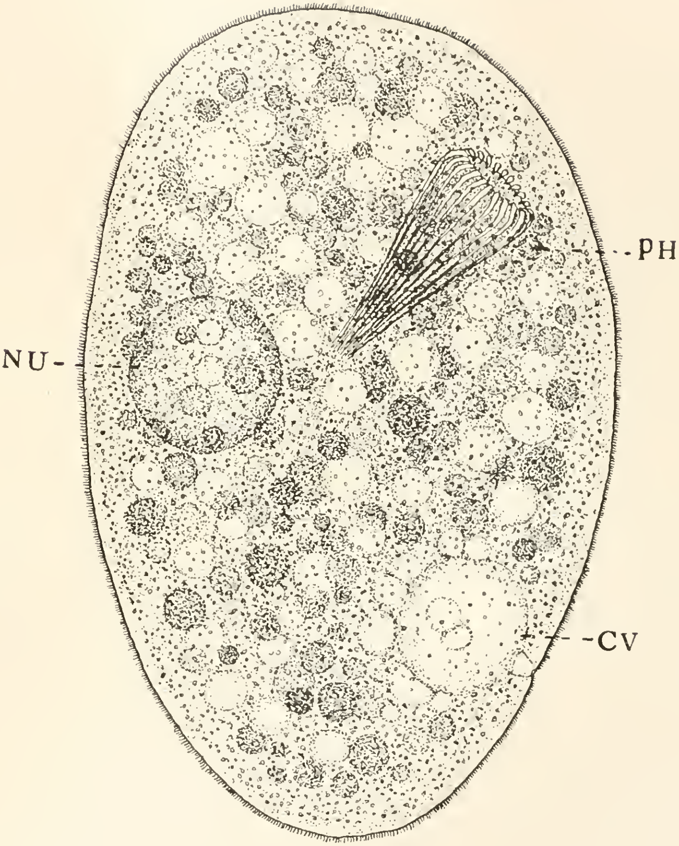
DESCRIPTION OF PLATE.

Right aspect of *Nassula* in the intermediate phase, *i.e.*, a *Nassula elegans* not yet sufficiently nourished to assume the larger, spheroidal contour of *Nassula ornata*. *PH*, pharynx; *NU*, nucleus; *CV*, contractile vacuole. × 250.



EDNA MACNALLY.

THE MORPHOLOGY OF THE COPULATORY STRUCTURES IN SOME CASES OF GYNANDROMORPHISM IN LEPIDOPTERA.

N. J. KUSNEZOV.

In spite of many excellent investigations on the gynandromorphism in Lepidoptera (Goldschmidt, 1914–1923; Cockayne, 1915, 1916; Meisenheimer, 1921; Morgan and Bridges, 1919; Poppelbaum, 1909, 1913; Gerould, 1925) and some ingenious speculations about its causes and origin, the perplexing phenomena of intersexuality and gynandromorphism are still far from being sufficiently elucidated. One may say that we now possess many facts, a good many hypotheses and visual schemes, but few experiments (Kosminsky, 1924) and a great lack of physiological interpretation, confined exclusively to the theory of sexual hormones.

Moreover, the facts, so numerous in this order of insects, are described insufficiently and based mostly on the superficial inspection of the external features, like the structure and pigmentation of wings and the structure of antennæ. The sexual organs have been examined in a few cases only. But the question of the correlation of these superficial, secondary sexual characters with the primary reproductive system is, of course, of very great interest.

In my previous paper [1] on this subject I have had occasion to describe in full the genital or copulatory apparatus of seven gynandrous individuals (*Pieris rapæ* L., *Gonopteryx rhamni* L., *Dendrolimus pini* L., *Pygæra timon* Hb. and three *Porthetria dispar* L.), halved or mosaic, and I came to the conclusion that "there is evident a considerable independence, in existence and evolution, of different structures of the sexual sphere in the Lepidoptera; this independence is most sharply defined between the gonads (primary) and the secondary (tertiary and so on) systems; but, on the other hand, the existence of a connecting influence between the secondary (copulatory) and tertiary (pigmentation, markings, etc.) sexual characters is also indisputable (cases of gynandromorphism)."

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[1] Kusnezov, N. J., "Contributions to the Morphology of the Genital Apparatus in Lepidoptera. Some Cases of Gynandromorphism," *Revue Russe d'Entomologie*, XVI., 1916, pp. 151–191.

The task of my present article[1] is to contribute further data regarding the dependencies and correlations between the copulatory organs and external sexual characters in lepidopterous gynandromorphs. I have had for examination five gynandrous specimens, all typically halved, viz., of *Argynnis paphia* L., *Gonopteryx rhamni* L., *Bupalus piniarius* L., *Malacosoma neustria* L., and *Lycæna argus* L., preserved dry and pinned.

I am indebted for this interesting material to the generosity of Messrs. A. P. Tshernyshev (Kaluga), A. I. Iljinskij (Kaluga), A. M. Djakonov (St. Petersburg) and V. J. Fridolin (St. Petersburg) who most kindly put it at my disposal.

Argynnis paphia L.—Wings: typical male on the right, valesina-female on the left. Left labial palpus female in shape and color. (Legs broken off.) Hairs on the thorax and abdomen fulvous or male on the right, and greenish-gray or female on the left side of the body. No observable differences in the antennæ. Captured at Kaluga.

Copulatory Apparatus (Figs. 2 and 3).[2] Nearly perfectly male. Valvæ, with their harpæ in the form of two hooked processes, as well as the processus superior and inferior and so-called crista obliqua, have nearly the same shape and size as in the normal male (Fig. 1). The differences seen in preparations, like the incisions and emarginations on the valvæ and the length of the processus inferior, or the size of the penis, are quite unimportant and lie within the limits of individual variability. The main abnormality in the copulatory apparatus of this specimen is confined to the tegumen, *i.e.*, to the complex of the 9th and 10th tergites fused together, and to the uncus, the dorsal appendage of the latter segment. The right side of the tegumen (Fig. 3, *tg.* 10 ♂) is weaker and less developed in comparison with that of the normal (Fig. 1, *teg.*), and, in particular, its uncus is small, reduced and deprived of the processes which adorn its dorsal surface and have the form of a cock's comb. Moreover, this uncoid is situated asymmetrically, being clearly displaced to the right side. In one word, this uncoid appears to be a right half

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[1] Read at the December meeting of the Russian Entomological Society in 1925.

[2] In the description of the genital armature I use the terminology elaborated by myself in my introduction to the study of Lepidoptera (Kusnezov, N. J., "Faune de la Russie. Lépidoptères. Introduction," Vol. I., 1915, published by the Russian Academy of Sciences).

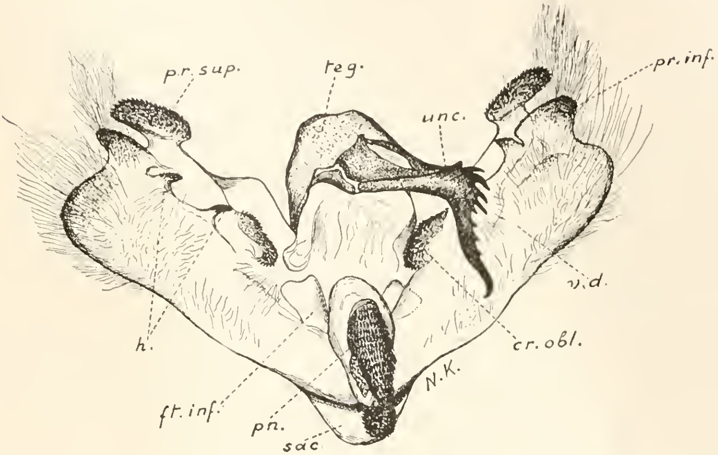

FIG. 1. Copulatory apparatus of the normal male of *Argynnis paphia* L.: *teg.*, tegumen; *unc.*, uncus; *pr. sup.*, processus superior valvæ of Petersen (cercina of Fruhstorfer); *pr. inf.*, processus inferior valvæ of Petersen; *h.*, harpæ (clinopus of Fruhstorfer); *ft. inf.*, fultura inferior; *pn.*, penis; *sac.*, saccus; *cr. obl.*, crista obliqua of Petersen; *v. d.*, valva dextra.—Zeiss, obj. a_3, oc. I.

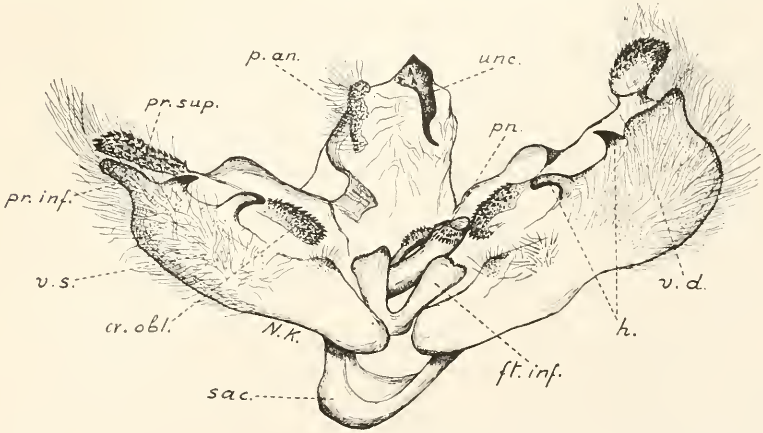

FIG. 2. Copulatory apparatus of the gynandrous *Argynnis paphia* L.: *p. an.*, papilla analis; *unc.*, uncoid; *pn.*, penis; *v. d.*, valva dextra; *v. s.*, valva sinistra; *h.*, harpæ; *ft. inf.*, fultura inferior; *sac.*, saccus; *cr. obl.*, crista obliqua; *pr. inf.*, processus inferior; *pr. sup.*, processus superior.—Zeiss, obj. a_3, oc. I.

only of the normal uncus. On the left side of the tegumen an elongate hairy prominence is situated, which, without hesitation, should be identified as the left papilla analis of the female (Fig. 2, *p. a.*). Lastly, on the pleural regions of the 8th and 9th abdominal segments there are some deformities rather exactly reproducing the pleural portions of the female abdominal segments (Fig. 3, *pl.* 7 ♀ and *pl.* + *st.* 7 ♀).

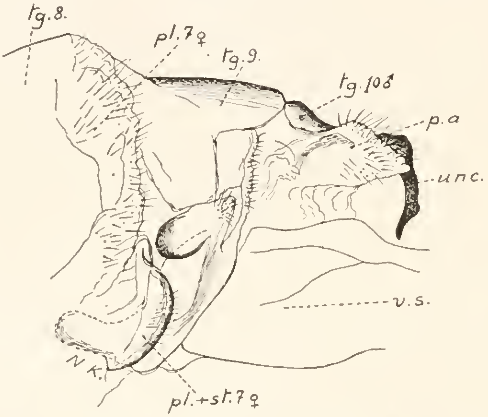

FIG. 3. Copulatory apparatus of the gynandrous *Argynnis paphia* L., side view: *tg. 8*, 8th tergite; *pl. 7* ♀, 7th pleurite of the female; *tg. 9*, 9th tergite; *tg. 10*♂, 10th tergite of the male; *p. a.*, papilla analis; *unc.*, uncoid; *v. s.*, valva sinistra; *pl.* + *st. 7* ♀, 7th pleurite and sternite of the female fused together.—Zeiss, obj. a_2, oc. I.

Thus, in this case, the structural and pigmental female characters of the wings, labial palpi and hair clothing show a correspondence with the copulatory apparatus on the same side of the body. In other terms, the tertiary sexual characters (structure and pigmentation of the wings and palpi) are strongly divided and separated by the symmetry plane, while the secondary ones (copulatory structures) are to some extent independent of this plane. Further, the male genital structures are markedly dominant and the female ones repressed.

Gonopteryx rhamni L.—Wings: female on the left, male on the right. (Antennæ broken off.) Both labial palpi yellow. No differences in the legs. Captured in the region under the Kaluga government.

Copulatory Apparatus (Fig. 4).—Valvæ, penis, with its vallum (Fig. 4, *v. p.*), as well as the saccus (Fig. 4, *sac.*) nearly normal.

Tegumen and uncus absent, and replaced by a pair of rather normal double papillæ anales of the female. Moreover, the bursa copulatrix, with its appendix (Fig. 4, *b. c.* and *a. b.*) and the lamina dentata (*l. d.*), are well developed and provided with a ductus bursæ which opens in the ostium bursæ (*o. b.*) lying normally, between the 7th and 8th sternites, but immediately on the left of the invagination of the saccus. Below this latter an androconial pouch (Fig. 4, *sin. and.*) is situated, representing in miniature the much larger structure of this kind in the normal female.

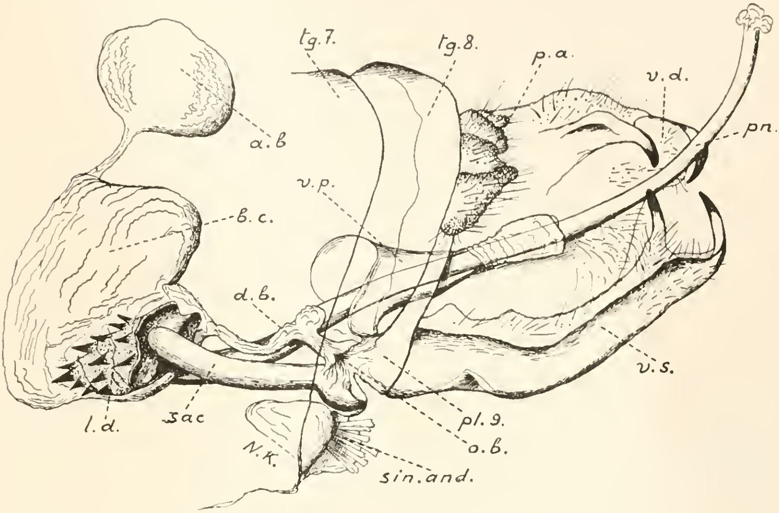

FIG. 4. Copulatory apparatus of the gynandrous *Gonopteryx rhamni* L.: *tg. 7.* and *tg. 8.*, 7th and 8th tergites; *p. a.*, papillæ anales; *v. d.* and *v. s.*, valva dextra and sinistra; *pn.*, penis; *pl. 9.*, pleurite of the 9th segment; *o. b.*, ostium bursæ; *sin. and.*, sinus androconialis; *sac.*, saccus; *l. d.*, lamina dentata; *b. c.*, bursa copulatrix; *a. b.*, appendix bursæ; *d. b.*, ductus bursæ; *v. p.*, vallum penis.—Zeiss, obj. AA, oc. I.

Thus, in this case we find in the structure of the copulatory apparatus no correspondence with the disposition of the wing pigments. The latter, male and female, are strongly divided and separated by the plane of bilateral symmetry, while the copulatory appendages are divided rather by a frontal plane, the female papillæ lying above, and male penis, saccus and valvæ below it. But at the same time the internal reproductive organs, the bursa copulatrix of the female with its appendages and, probably, the ductus ejaculatorius of the male exist side by side, with their openings in normal position.

Malacosoma neustria L.—Wings: female on the left, male on the right. Antennæ: right male, left female. Specimen captured in the region under the Kaluga government.

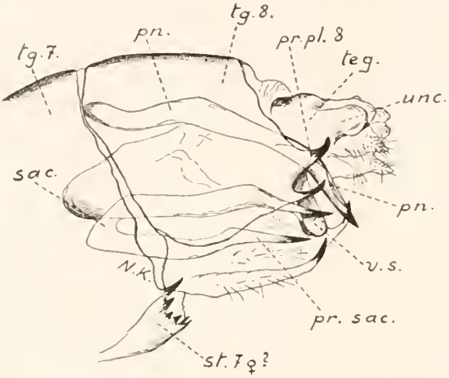

FIG. 5. Copulatory apparatus of the gynandrous *Malacosoma neustria* L., lateral view: *tg. 7.* and *tg. 8.*, 7th and 8th tergites; *pn.*, penis; *pr. pl. 8.*, processus of the 8th pleurite; *teg.*, tegumen; *unc.*, uncus; *v. s.*, valva sinistra; *pr. sac.*, processus sacci; *st. 7* ♀ ?, 7th sternite of the female (probably); *sac.*, saccus.—Zeiss, obj. AA, oc. I.

Copulatory Apparatus (Figs. 5 and 6).—Almost normal male, except the hind margin of the 7th abdominal sternite which bears slightly stronger indentations than those in the normal male. The female structures absent.

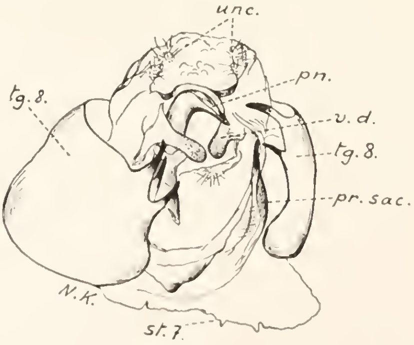

FIG. 6. Copulatory apparatus of the gynandrous *Malacosoma neustria* L., ventral view; abbreviations the same as in Fig. 5.—Zeiss, obj. AA, oc. I.

Thus, in this case, the pigmental and structural sexual differentiations of the wings and structural sexual features of the antennæ, strongly divided by the plane of bilateral symmetry, do not correspond with the differentiation of the genital armature.

Bupalus piniarius L.—Wings: left fore wing ill-developed, curled; left hind wing not developed and rudimentary; on the right side the fore and hind wings normally developed. Color of all wings pale yellowish, more feminine than that of the yellowish forms of males; but, as far as appearances go, wings on both sides

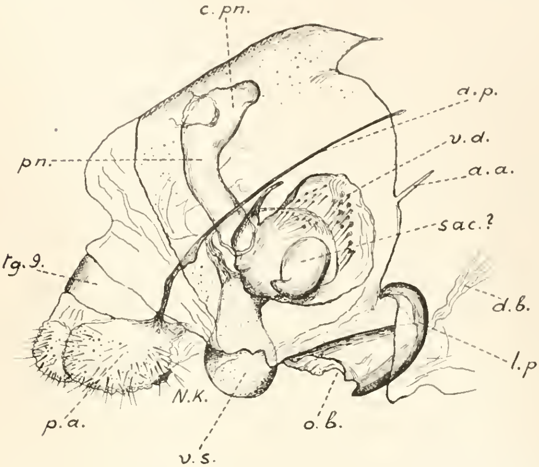

FIG. 7. Copulatory apparatus of the gynandrous *Bupalus piniarius* L., right side view: *c. pn.*, cœcum penis; *a. p.*, apophyses posteriores; *v. d.* and *v. s.*, right and left valvoids; *a. a.*, apophyses anteriores; *sac.?*, saccus (probably); *d. b.*, ductus bursæ; *l. p.*, lamella postvaginalis; *o. b.*, ostium bursæ; *p. a.*, papillæ anales; *tg. 9.*, 9th tergite; *pn.*, penis.—Zeiss, obj. AA, oc. 2.

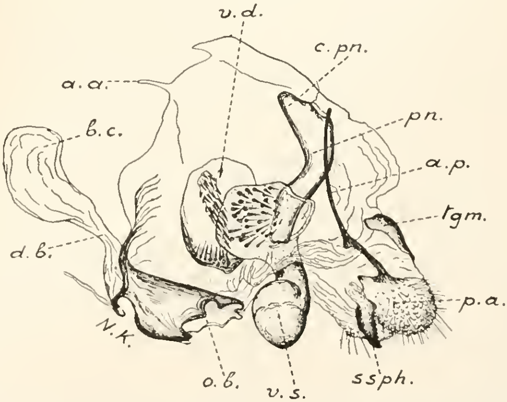

FIG. 8. Copulatory apparatus of the gynandrous *Bupalus piniarius* L., left side view: *v. d.* and *v. s.*, right and left valvoids; *c. pn.*, cœcum penis; *pn.*, penis; *a. p.*, apophyses posteriores; *tgm.*, tegumen; *p. a.*, papillæ anales; *ssph.*, subscaphium; *o. b.*, ostium bursæ; *d. b.*, ductus bursæ; *b. c.*, bursa copulatrix; *a. a.*, apophyses anteriores.—Zeiss, obj. AA, oc. I.

are uniform in color and markings. Antennæ: left bipectinated, male; right filiform, female. Bred specimen, emerged at St. Petersburg January 27, 1901.

Copulatory Apparatus (Figs. 7 and 8).—8th and 9th segments female, with nearly normal apophyses anteriores and posteriores (Figs. 7 and 8, *a. a.* and *a. p.*). Papillæ anales well developed, symmetrical. Ostium bursæ quite normal, strongly chitinized. Bursa copulatrix normal, but lamella antevaginalis absent, the postvaginalis in the form of a chitinous band (Fig. 7, *l. p.*). On the other hand, there is a penis, with its cœcum (Fig. 7, *c. pn.*), rather well developed and lying inside of the body cavity, but capable of being protruded through a very narrow orifice in the diaphragm. Uncus absent. Rudiments of tegumen in the form of a more strongly chitinized left portion of the 9th tergite (Fig. 8, *tgm.*). A well-developed left portion of the subscaphium (Fig. 8, *ssph.*) lying below the left papilla analis. As to the valvæ, they are both present, but deformed, lying within the body cavity and protruding only partially outside (Figs. 8 and 9, *v. s.* and *v. d.*). Lastly, an oviform structure lying freely in the body cavity on the external surface of the right valvoid (Fig. 7, *sac.*?) may be regarded as a rudiment of the saccus.

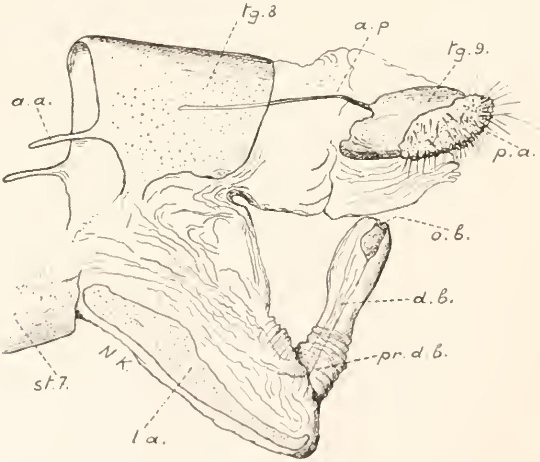

FIG. 9. Copulatory apparatus of the normal female of *Lycæna argus* L.: *tg. 8.* and *tg. 9.*, 8th and 9th tergites; *a. p.*, apophyses posteriores; *p. a.*, papillæ anales; *o. b.*, ostium bursæ; *d. b.*, ductus bursæ; *pr. d. b.*, processus ductus bursæ; *l. a.*, lamella antevaginalis; *st. 7.*, 7th sternite; *a. a.*, apophyses anteriores.—Zeiss, obj. AA, oc. I.

Thus, in this case almost all male and female genital structures, except the male uncus, coexist, though the male ones are deformed; they are disposed, with the exception of the rudimentary subscaphium, on both sides of the plane of bilateral symmetry. This case is similar to that of the *G. rhamni* L. in the respect that here also the paired female papillæ and the paired male valvæ are divided from one another by the frontal plane.

Lycæna argus L. (*ægon* Schiff.).[1]—*Wings:* male on the left and female on the right. Antennæ uniform. Both fore legs with female tarsus.

Copulatory apparatus (Fig. 10) predominantly female. Tergite and sternite of the 8th segment nearly normal except for the distal angles of the tergite being obtusely shortened. 10th tergite split on its dorsal surface. Apophyses anteriores and posteriores, as well as papillæ anales, normal. Membranous tube (Fig. 10, *pr. d. b.*) bearing on its tip the ostium bursæ, developed, but about three times stouter than normal. A pair of lamellæ antevaginales a little wider and shorter than normal ones. Ostium bursæ present, its ductus indistinct. Bursa copulatrix bag-like, long, plicated, nearly normal. A deformed, strongly chitinized, plate lying in the body cavity between the 8th and 9th tergites may be considered as male valvoid, because on its distal extremity it

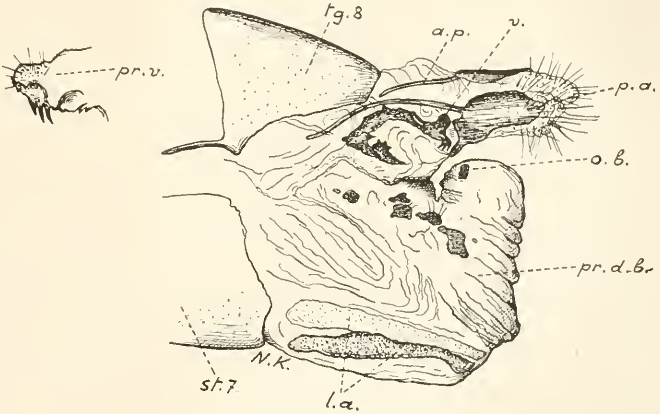

FIG. 10. Copulatory apparatus of the gynandrous *Lycæna argus* L.: *v.*, valvoid; *pr. v.*, processus valvæ of the normal male; other abbreviations as in Fig. 9.—Zeiss, obj. AA, oc. I.

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[1] Species with claws on the fore tibiæ.

bears a process having three teeth quite similar to those on the tips of the normal valva (Fig. 10, *pr. v.*). Under this valvoid there are some minute sclerites disseminated over the intersegmental membrane of the tube of the ductus bursæ. From their consistence and structure they also may be considered as crushed rudiments of the male valva.

Thus, in this case we observe a predominance of female characters in the form of both fore tarsi and almost all parts of the copulatory apparatus which are but slightly deformed. Male features, represented only by rudiments of valvæ, are nevertheless situated on the same side of the plane of bilateral symmetry as the male wings.

In all described gynandrous individuals, however similar in their external appearance which is that of common halved gynandromorphs, quite different interrelations in the degree of development and disposition between the secondary and tertiary[1] sexual structures are observed. My present observations, as well as previous (1916), mentioned above, permit me, I think, to draw the following conclusions.

The division of the tertiary sexual structures into two halves by the plane of bilateral symmetry may be associated:

(1) With a division of the paired copulatory structures by this plane into two pretty normal halves, the unpaired organs, like penis, bursa and their ostia being developed nearly normally; the case of *Pieris rapæ* L.;

(2) With a more or less complete unisexuality of the genital structures: male, as in my cases of *Malacosoma neustria* L. (complete unisexuality) and *Dendrolimus pini* L., or female, as in *Lycæna argus* L.;

(3) With an intermediate position, where the structures of one sex are intermingled with those of the opposite sex on both sides of the body; these are the cases of *Pygæra timon* Hbn., *Bupalus piniarius* L., *Gonopteryx rhamni* L., one sex being rather more strongly developed than the other;

(4) With a division of the paired copulatory organs in respect to a frontal plane of the body, with the presence of the unpaired

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[1] Under the term of secondary sexual structures is meant the copulatory organs or genitalia, under that of tertiary the locomotor apparatus, pigments, sense organs and their accessories.

ones in a rather normal state; these are the cases of *Bupalus piniarius* L. and *Gonopteryx rhamni* L.

No case of purely crossed gynandromorph, *i.e.*, having the tertiary characters of a given sex situated precisely on the sexually opposite side of the body, has been, as far as I know, yet observed.

It is doubtful whether attempts to elucidate these various combinations by referring to their connection with the geometrical planes of the organism will have any success. The plane of bilateral symmetry, as is true of each other plane of the body, is by no means a real physiological factor; and physiology has nothing to do with these abstract morphological concepts. Very possibly, in general, no architectonic rules are followed by the developing organism. That is why the chimæra hypotheses fail also totally in their attempts to explain the confusion observed in the distribution of the secondary genital rudiments in certain gynandromorphs. Far more probable is the explanation (Morgan 1922) of these irregularities by a simple anatomical shifting of the diversely determined body cells during the embryonic development. So is also the hypothesis of the precocious development of one sex tissue in comparison with the other, growing across the plane of bilateral symmetry and encroaching upon another half differently determined (Gerould, 1925; partly Goldschmidt). The cellular and blastomeric hypotheses (Boveri, 1888, 1902, 1915; Lang, 1912; Cockayne, 1915) fail also to explain the multitude of facts when the body planes are nearly never maintained but are in different ways transgressed. Lastly, it is a rather discordant fact for the hypothesis of polyspermy (Morgan, 1905, 1909) that only a quite insignificant number of cases of the plurality of the same organ have ever been observed.[1] The generally accepted hormonal theory of the chemical stimulation also meets great difficulties in the explanation of asymmetrical, halved, gynandromorphism connected with the body planes.

My opinion is that the phenomena of the "irregular" spacial distribution of sexually determined parts of the body should be interpreted as cases of organic regulation (in the sense of Driesch) in which the "normal" conditions are altered in the gynandrous embryo by the interruption of the normal chemical stimulation of somatic cells which have already obtained their sexual determination.

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[1] Those described by Goldschmidt (1921) need further examination. A "supernumerary" right valva described by Gerould (1925), more than possibly, will prove to be but a deformed portion of the right valva.

It is a custom to unite all sexual structures, other than gonads, under a general term of "somatic" structures and to subordinate them to the hormones of the gonads. But (1) for insects this subordination is yet to be proved (Oudemans, 1898; Meisenheimer, 1909; Kellogg, 1904; Kopeč, 1908–1913); and (2) it is evident that the somatic sexual structures are to be classed among several rather independent groups, each group being influenced by its own regulating factor, or, perhaps, possessing its own determination (Abderhalden, 1911). The idea of a sexual determination preexisting in all somatic cells grows more and more acceptable.

But, on the other hand, the division of the reproductive system into primary, secondary organs, and so on, can be admitted only theoretically. Physiologically speaking this system is a unity. Perhaps, we should not speak, therefore, of dependencies of one group upon another, but only of the degrees observed in the display of the whole system in its parts collectively or individually. And, it may be, the sexual chemical factors, though different in their manifestations, are in reality the same, differing only quantitatively.

Zoölogical Museum of the
Russian Academy of Sciences,
May 18, 1926.

A NOTE ON THE OCCURRENCE AND HABITS OF A LUMINOUS SQUID (*ABRALIA VERANYI*) AT MADEIRA.

S. STILLMAN BERRY,

REDLANDS, CALIFORNIA.

Originally made known in very incomplete fashion (vide Rüppell, 1844) from individuals taken in the neighborhood of Messina, our knowledge of this extremely interesting species of squid is based on the information gleaned from the very limited number of specimens which have now and then fallen into the hands of students of the group, principally systematists, since that time. These specimens have been entirely Mediterranean in origin, the majority of them, like the type, from Messina, but a few from Nice, Toulon, and so on. Until quite lately it has happened that almost every captured specimen has found its way into the collections of one or another of the German museums. It has therefore somewhat curiously come about that whereas neither Gray three quarters of a century ago, nor Tryon, nor even Hoyle seem to have had any actual material of the species to work upon, Pfeffer's recourse to the Hamburg and other German collections, especially that at Leipzig, yielded him no less than fifteen specimens (Pfeffer, '12, p. 136). More recently some further specimens, likewise taken at Messina, have been the subject of a short but beautiful memoir by Dr. Silvia Mortara ('22) on the histology of the photophores. Practically the whole of our real knowledge regarding *Abralia veranyi*, from whatever aspect, is to be found in this paper, and in the monograph of Pfeffer, although there is some information to be had from an older paper by Steenstrup ('80) and another by Joubin ('95), where the luminous organs of some specimens from Nice which are probably referable to this species are described under the name *Abralia Oweni*.

It is of interest to remember that of the seven described species of *Abralia*, *A. veranyi* as at present understood is the only occidental one. One species comes from the Red Sea. The remainder are all Indo-Pacific, two of them being Hawaiian. From the waters of the western hemisphere, even in what would seem entirely appropriate latitudes, the genus is as yet unknown. Thus from the entire Atlantic beyond the gates of the Mediterranean no member of the genus has been reported hitherto.

Furthermore in regard to the behavior of the animal in life or concerning even so much as its appearance at the time of capture I have been able to discover no published information in as nearly an exhaustive search as has been possible to me. What we know or think we know of the natural history of this interesting animal is almost entirely inferential or presumptive.

In view of the situation outlined it is therefore a matter of no small satisfaction to be able to report not alone a considerable and important extension of the known geographic range of this genus and species, but to record a few observations made by the captors of the specimens, which throw a certain light on the habits of the animal, are a welcome contribution after the nearly blank record of the past eighty years, and are themselves withal full of lively interest. For this my acknowledgment is due to the gentlemen to be indicated, as well as to others later mentioned in the course of this paper.

When Professor T. D. A. Cockerell of the University of Colorado returned to the United States from his Madeiran trip in 1921, he brought with him various specimens of invertebrates, among them as by no means the least of the treasures, three small squids. These he generously turned over to me, with the word that they were given him by Senhor A. C. de Noronha of Funchal. The bottle containing them bore the following label in Sr. de Noronha's hand: "Luminous. Caught during the night near the shore rocks. Funchal. June 19, 1917." The three specimens proved to include a beautifully preserved male and two females of the rare Mediterranean enoploteuthid referred to in our preface, *Abralia veranyi*. Through the kindness of Dr. Silvia Mortara I have also been the fortunate recipient of one of her precious Messina specimens, captured in November, 1921. A direct comparison of the Funchal form with authentic Mediterranean material has therefore been made possible.

The principal references covering this species in the literature are given below. Those who desire a more complete synonymy are referred to Pfeffer, '12.

Abralia veranyi (Rüppell, 1844).

(Dates of more important references italicized.)

1844. *Enoploteuthis Verany* Rüppell.—Giorn. Gab. Messina, 26, p. 3, f. 2.

1851. *Enoploteuthis Veranyi* Vérany.—Céph. médit., p. 83, pl. 30, f. b.

1879. *Enoploteuthis Veranyi* Tryon.—Man. Conch. (1), v. 1, p. 173, pl. 76, f. 318–319 (after Vérany).

1880. *Abralia veranii* and *Veranyi* Steenstrup.—Overs. K. D. Vid. Selsk. Forh. 1880, p. 92, 110 [22, 40], pl. 3, f. 2–6.

1886. *Abralia veranyi* Hoyle.—Ceph. Challenger Exp., p. 38, 217 (merely catalogued).

1895. *Abralia Oweni* Joubin.—Mém. Soc. Zoöl. France, v. 8, p. 220 [9], f. 6–11 (photogenic organs).

1899. *Abralia Veranyi* and *Enoploteuthis Verany* "Rüppel" Ficalbi.—Monit. Zoöl. Ital., v. 10, p. 80–82, text f. 2 (after Rüppell).

1900. *Abralia armata* (pars) Pfeffer.—Synops. œgops. Ceph., p. 167.

1900. *Asteroteuthis veranyi* Pfeffer.—Teuthol. Bemerk., p. 289.

1912. *Asteroteuthis Veranyi* Pfeffer.—Monogr. Œgops., p. 129, 785, 794, pl. 16.

1912. *Abralia Veranyi* Pfeffer.—*id.*, p. 785, 794.

1922. *Abralia veranyi* Mortara.—R. Com. Talass. Ital., Mem. 95, p. 1–20, text f. 1–2, pl. (photogenic organs).

A careful comparison of the Madeiran specimens with that from Messina and with the lengthy account given by Pfeffer has brought to light no points of difference thought to be in any respect essential from the taxonomic standpoint. As will be seen from the appended table of measurements, the dimensions of the Madeiran examples are as a rule well in excess of those of the individual from Messina, but this is very decidedly more true of the two females than of the single male and may possibly prove to be, in part at least, a secondary attribute of sex. The wider, more flaring mantle possessed by all three Madeiran examples is doubtless but an incident of the mechanics of their preservation. The same is doubtless true of the apparent great number of chromatophores resulting in the consequent darker color of these specimens. The greater conspicuousness of the chromatophores seems to carry a concomitant accentuation of the photophores so that at first sight they appear much more numerous than in the Messina specimen. The attempt to count the photophores in corresponding areas, however, has not led to proof of any actual critical difference in their number.

The male indeed does show a few slight differences in the structure of its beautifully preserved hectocotylus, especially in the fact that the tip of the modified arm is so produced and attenuated beyond the curious fleshy folds which are a feature of the distal part of this arm in the present species, and regarding the exact function of which we are still quite in the dark except that there seems good reason to believe that they may serve in some fashion or other in the manipulation and fixation of the spermatophores.

This male and one of the females show but three hooks on each tentacle club. The other female has three hooks and a possible remnant of a more minute proximal one on the left club, while the right club bears four hooks in agreement with both clubs of the Messina specimen. These seem but normal variations. I have noticed no record of any specimens with clubs bearing less than two nor more than four hooks.

A certain degree of sexual dimorphism is, as already noted, indicated by the present specimens, but it is not conspicuous, being chiefly manifested by the somewhat smaller body and fins of the male. The differences noted appear both absolute and relative. The fins are almost equally wide in proportion to mantle length in both males and females, but in the former they are relatively somewhat shorter, giving an index of about 60 as against 66 in the females. The arms of the male on the other hand are somewhat longer in relation to the body than those of the female.

As was discovered by Steenstrup ('80, p. 110), the spermatophores become attached in a rosette-like cluster to the inner surface of the mantle of the female, this being in the median line just back of the nuchal cartilage. These clusters are very conspicuous in both of the females before me, but there is present in each of them in addition to the principal rosette, a much smaller cluster of 4 to 8 spermatophores or remains of the same adhering to the visceral mass at its mesial junction with the collar, a position not quite opposite to that occupied by the main rosette, but more anterior. Possibly therefore this smaller cluster is to be regarded as a fragment of the larger one, adhering here accidentally at the time of the emplacement of the latter. In both of these specimens the ovaries are swollen and packed with developing ova. Just how soon the ova would be ready for extrusion is at present problematic.

TABLE OF MEASUREMENTS.

Locality	Messina.	Funchal.		
Sex	♂	♂	♀	♀
	mm.	mm.	mm.	mm.
Total length	110	115	120	130
Length of body, dorsal	40	40	45	46
Length of body, ventral	38	37	41	43
Tip of body to base of dorsal arms	54	52	61	60
Width of fin at widest point	14	17	17	18
Length of fin	23	24	30	30
Width across fins	33	37	38	42
Width of body	13	18	18	18
Depth of body	12	13	13	15
Width of head across eyes	15	12	11	14
Length of head (nuchal cartilage to base of dorsal arms)	13	12	15	15
Length of funnel, median	9	9	8	10
Length of right dorsal arm	21	29	28	26
Length of left dorsal arm	23	30	28	26
Length of right second arm	25	35	33	31
Length of left second arm	27	34	33	30
Length of right third arm	24	33	31	31
Length of left third arm	25	32	32	28
Length of right ventral arm	29	32	32	29
Length of left ventral arm	29	32	32	29
Length of right tentacle	54	62	62	68
Length of right tentacle club	9	9	11	11
Length of left tentacle	55	63	59	69
Length of left tentacle club	10	9	9.5	11
Length of hectocotylized part of left ventral arm (taken from last hook to tip)	8	10	—	—

The male shows a large spermatophore bundle in process of extrusion from Needham's sac and it may be added that the same observation is true of the male from Messina.

Upon writing to Senhor de Noronha and to Senhor Adão d'Abreu Nunes, to whom, I believe, belongs the credit for the actual capture of the specimens, these gentlemen courteously responded with notes of so great intrinsic interest that it seems desirable by means of a somewhat free translation to publish them in full, the more especially as direct observations on the luminosity of cephalopods in life under natural conditions are still of exceeding rarity.

The following excerpt is in free translation from a letter from Sr. de Noronha under date of November 13, 1921.

> "In reading my notes, I find that the cephalopod was captured by a friend, a great fish enthusiast, toward midnight of the 19th of June, 1917, at the surface of the sea and in the artificial harbor of Pontinha, to the west of Funchal.

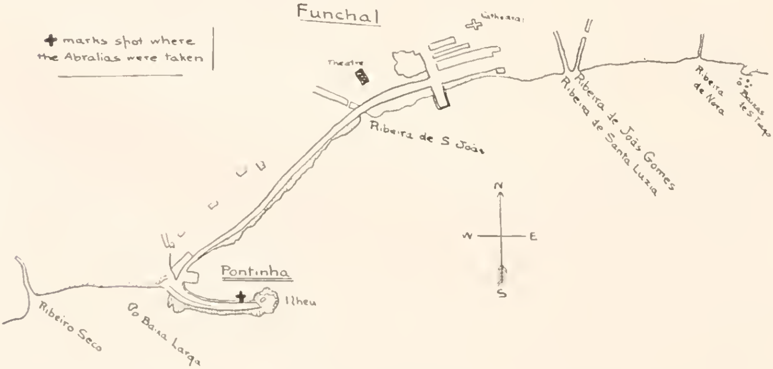
Funchal
✚ marks spot where the Abralias were taken
Cathedral
Theatre
Ribeira de S João
Ribeira de Joâs Gomes
Ribeira de Santa Luzia
Ribeira de Nera
Baixas de S Tiago
N
W
E
S
Pontinha
Ilheu
Ribeiro Seco
Baixa Larga

FIG. 1. Sketch map of the Bay of Funchal. The point of capture of the specimens of *Abralia veranyi* mentioned in the text is marked by a +.

> "I was there on the quay myself that selfsame evening, and I was very happily able to record that the animal was luminous, the light being very vivid and of a lovely ultramarine blue. One saw 5 lights ('*foyers*'), disposed in an arc around the eye and on the lower region of this organ, 2 of these photophores being larger and 3 smaller. On the body, the head, and the arms there were many similar lights, which were more numerous on the ventral side. I also noticed that out of the water the animal was still very lively and tried to bite in its anger.
>
> "From the jetty was perceived from time to time an indi-

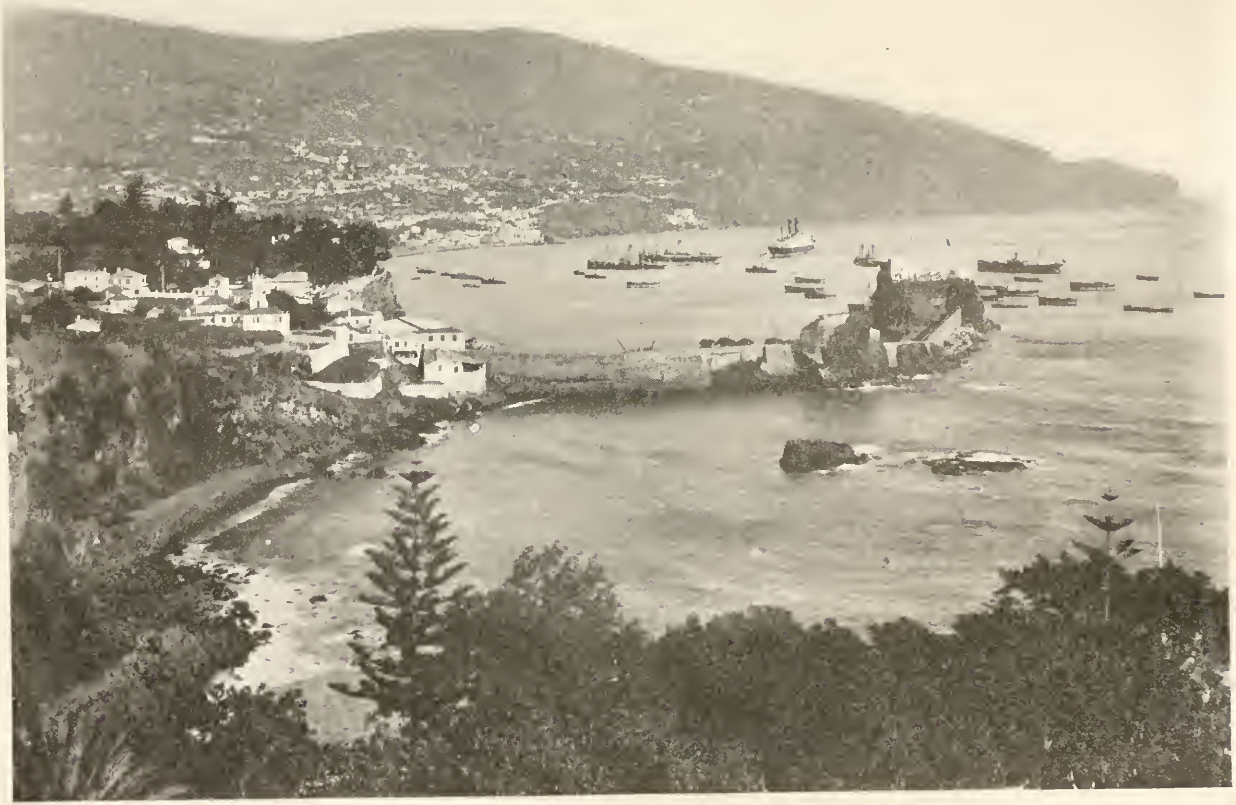

FIG. 2. The Bay of Funchal and harbor of Pontinha viewed from the west. (Photograph by M. O. Perestrello & Fos., Madeira.)

> vidual which swam rapidly near the surface, giving out its phosphorescence, and then my friend from the top of the wall dexterously manipulated his long-handled 'peneiro' (dip-net). I obtained in this manner a dozen examples, and I could have had many more if I had had need of them. It was an evening warm and calm, and it seems to me that it is for this fine weather that the *Abralia* shows preference. At least it is in summer and autumn that the charming cephalopod arrives near the walls of the harbor of Pontinha. This locality is very sheltered, almost closed to currents and winds, and it is perhaps this circumstance which draws it to this nook of the shore, attracted furthermore by the lamp which illumines the quay. Indeed, as I believe, this cephalopod has never been seen in the five less protected places on the south coast of Madeira, where, during the night, they drag the fish seines, and where it would be easy to capture them if they were there. I myself have investigated these places, but I have seen taken in the nets only the common cephalopods of Madeira: a *Loligo*, a *Sepia*, and a *Polypus*.
>
> "I would also broach the opinion that the *Abralia veranyi* is an abyssal species which at night in summer migrates vertically and horizontally to attain the shore line, and by day betakes itself anew to the depths of the ocean."

Under date of February 1, 1922, Sr. Nunes wrote me further:

> "The cephalopod in question has been captured by myself in the sheltered quay of this city of Funchal, called the Quay of Pontinha, during the months of July, August, and September.—Almost every year one may capture them in this harbor during the night where they approach the steps of debarcation, following the lighting of the electric lamps of the above-mentioned steps. With a certain alacrity one may catch them with the aid of a little wire basket, because these animals come almost to the surface of the water, being distinguishable by the brilliancy ('éclat') of a bluish phosphorescence which they cause to gleam from their eyes."

From these notes it would appear that the phenomena described are distinctly seasonal in character. This fact, the exceeding vigor and activity evinced by the animals when captured, and finally my own observations on the physiological condition of both males and females, affords convincing evidence that the inshore nocturnal migration of the Madeiran *Abralia* is essentially of reproductive significance and has to do with either the mating or the spawning, or very possibly indeed with both. Such a conclusion finds the strongest possible confirmation in the detailed observations of Ishikawa ('13) and Sasaki ('14) on a somewhat nearly allied species of squid, *Watasenia scintillans*, the famous Firefly Squid ("Hotaru-ika") of Japan. The parallel extends to still further particulars, but so little information of consequence concerning the habits and life histories of the smaller squids has accumulated that the Japanese observations are almost the only ones of any relevance to be found in the literature. Like the *Abralia*, *Watasenia* "is a deep-sea animal, living during the day in a depth of 100 or more fathoms, and when the night is at hand, they approach to the coast, and after sunset they lay the eggs, and as soon as they finish their spawning, go back to the deep sea" (Sasaki, '14, p. 95). After the fixation of the spermatophores in the nape of the female (in quite a different position, as it would appear, from what is to be observed in *Abralia veranyi*), the male *Watasenia* is thought to perish. The season of this extraordinary migration varies somewhat in different parts of the Japanese Empire, but in Toyama Bay on the west coast it is late April and May. Here the firefly squid comes inshore in such enormous numbers that their fishery is a considerable industry, the total catch being given by Sasaki as 1,000 tons. Due to the disappearance of mated males, nearly the entire catch seems to be comprised of females (one count given is 1 ♂ to 79 ♀ ♀). No juvenals are found with them, and only a small per cent. of those taken were found to have food in the stomach. A net drawn up at 9 or 10 P.M. is said to be better filled than one hauled in at 3 or 4 A.M., a circumstance which Sasaki suggests may be largely due to the fact that the schools swim in from the deep when sunset approaches, lay their eggs towards evening, and become entrapped in the nets on their way back. The fact that the *Abralia* is to be seen soon after the lights on the quay are illuminated may indicate that something of a nearly similar nature goes on here.

Sr. de Noronha's allusion to the enraged state of the animal when captured is curiously in accord with the behaviour of *Watasenia* at the height of its own (spring) migration, at which time Sasaki states that "they attacked us violently, biting our hands with their jaws." Such as the Japanese catch in late summer or autumn, on the other hand, are quiet and show little vitality.

From the relative emphasis laid upon the sources of the animal's illumination by both Madeiran observers, but especially by Nunes, it would seem that the ocular photophores of this *Abralia* irradiate a conspicuously brighter light than the more abundant organs of the body surface. This is very much what one would superficially expect from the general appearance of these organs in the dead animal. Turning again to the Japanese species we find that Ishikawa ('13, p. 168) likewise states that of the three types of photophores found in *Watasenia scintillans*, at least two of which are entirely homologous with those of *Abralia veranyi*, the organs of the outer integument come last in the intensity of their light. Yet it must be remembered further that Sasaki, working more extendedly on the same species, was unable to make out any difference between the light of the ocular and that of the integumentary photophores. However this may be, the function of light production in both species would seem to be essentially the same. The brilliance of the display stressed by both Madeiran and Japanese observers, coinciding as it does in each case with the schooling habit, the nocturnal migration, and the period of sexual activity, is most readily interpreted as a mating phenomenon, at least in very large part. I do not mean by this that the sexes actually recognize one another's different nature by means of corresponding differences in the luminosity of male and female, although at the same time the possibility of such recognition should not be too quickly excluded from consideration merely because Sasaki, who inquired into this aspect of the question quite particularly, found himself entirely unable to distinguish the sexes merely by the light of the animals at night. For there is another important way in which the photogenic function could serve an animal behaving as *Abralia veranyi* does during the reproductive season, and that is by simply furnishing a visual method by which the schools can assemble or keep together during the vicissitudes of migration. If this be supplemented either by slight visible sex differences, or by some chemical or other means of inter-attraction, such an arrangement might be sufficiently adequate to insure the maintenance of the species. Further field observations with this point in mind would doubtless yield some valuable and entertaining information.

At first thought it seems passing strange that so conspicuous a phenomenon has not been observed more commonly. However, for the occurrence of species possessing such habits at stations convenient for observation near the shore there would seem to be required not alone shelter from heavy waves but an abrupt slope from the shore-line to the 100-fathom mark so that the contingent requirement for lateral migration be not too great, or similarly a near approach on the part of the deeper regions of the sea either by the agency of a submerged valley or some other considerable depression. Such conditions are perhaps not always found in appropriate combination in the regions inhabited by these species. Whatever the said conditions may be, Funchal evidently satisfies them, and so affords nearly ideal opportunity for the study of *Abralia veranyi.* It is hoped that possibly this will prove by no means the only enoploteuthid squid to be found there.

Whether the animal occurs in the near neighborhood of Madeira at all seasons of the year, or whether its nightly appearance in summer is but the visible culmination of a more extended series of migrations from much farther afield, is a final interesting problem which must be left for some future deep-sea expedition to solve.

Acknowledgment is due to Senhores M. O. Perestrello & Fos. of Madeira for the use of the accompanying photograph, and to Sr. de Noronha for the original of the small sketch map appended. A grant from the Rumford Committee has also been a contributing factor in the preparation of this report.

SUMMARY.

1. *Abralia veranyi* (Rüppell) is a somewhat rarely captured Mediterranean cephalopod, possessing interesting luminous qualities due to the presence of numerous and exceedingly complicated photogenic organs.

2. It is here reported from the Bay of Funchal, Madeira, this constituting, it is believed, the first record of the species from the open Atlantic.

3. The specimens appear to differ in only very minor respects if at all from those of Mediterranean origin.

4. The species comes into the shallow water of the harbor at night during the summer months, supposedly for the purpose of mating, spawning, or both.

5. Owing to their comparative abundance at this time of year and the ease with which they may be observed and captured, Funchal is evidently an unusually favorable locality for the study of the light production or general bionomics of this interesting little squid.

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CONVERGENCE OF COLORATION BETWEEN AMERICAN PILOSE FLIES AND BUMBLEBEES (*BOMBUS*).

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The European and Asiatic Group of *Volucella Bombylans*.

In a previous paper, in which the heredity of color variations in the European fly *Volucella Bombylans* has been described, an attempt has been made to show the striking resemblance in coloration between various flies of the families *Syrphidæ*, *Asilidæ*, *Tabanidæ*, *Œstridæ* and *Bombylidæ* with various bumblebee species which inhabit the same geographical regions.

The study of those analogous color patterns which appear both amongst flies and bumblebees is interesting from a genetic point of view and bears on the problem of mimicry in animals.

The black *V. Bombylans* (Pl. I., Figs. 1, ♂, ♀ ; 2, ♀ ; 3, ♀) with its fulvous bands on the last abdominal segments has exactly the same coloration as the common European *Bombus lapidarius*, *B. confusus*, *B. rajellus*, *B. mastrucatus*, etc. The other variety, *V. hæmoroidalis* (Pl. I., Figs. 4, 5), is almost indistinguishable from many other *Bombus* species, as for example *B. agrorum*, *B. vorticosus*. The third variety of the *V. Bombylans*, the *V. B.* var. *plumata* (Pl. I., Figs. 6, ♂, ♀ ; 7, ♀) with its characteristic white hairs on the last abdominal rings, is extremely like *B. hortorum*, *B. lucorum*. *Volucella bombylans* (and its varieties), if really protected by her mimetic coloration, may be supposed to creep unobserved into a *Bombus* nest to lay her eggs there on the wax-combs. More often however the fly puts her eggs on the grass and moss, which covers the *Bombus* nest. This of course can only be performed with a *Bombus* species which builds its nest on the ground, not under the ground. In localities where many *Bombus* colonies of this kind are present the *Volucella* flies are also found in large quantities. The larvæ of *Volucella* feed on wax, pollen and decayed or injured *Bombus* larvæ (fresh larvæ are never attacked).

The "Mimicry" of *Volucella* offers a fascinating field for research and one of the steps which must be taken in the preliminary study of this case is to find out how close a resemblance there is between a variety of *Volucella* and the various species of *Bombus* in the same geographical area. Such a resemblance has been described in my previous paper for the European group of *Volucella*. I was interested therefore to see if this was true for the American varieties of *Volucella*.

Before dealing with this question I wish to refer to some new details on the coloration of the European *Volucella Bombylans* which must be also taken into consideration in the study of the North American group. Many flies of these species have been collected recently in the central parts of Russia, to see if there are any other variations than those which have been generally described. Specimens of *V. Bombylans* have been found with an entirely rufous abdomen (Pl. I., 2, ♀), which corresponds exactly to the color of the abdomen of the northern *B. laponicus* and other allied species. This variety has been collected in Russia only twice in a region where the *B. laponicus* does not occur. Another variety of *V. Bombylans*, which may be named *V. Bombylans* var. *flava*, is not uncommon. This fly has a spot of yellow hairs on the thorax (Pl. I., Fig. 3, ♀), but the rest of the fly is typical *Bombylans*. (Only females of this type have been collected.) The yellow spot of hairs of this black variety is located in the same place as the black spot of yellow varieties of male *V. hæmoroidalis* and male *V. plumata*. It is significant from a genetic point of view that in the European *Volucella* group of flies only this little area of thoracic hairs is limited to one sex, whereas the entire body coloration is otherwise alike in both sexes. Thus the yellow spot of the black variety seems to appear only in the females, the black spot of the yellow varieties on the contrary is limited to the males (*V. plumata* and *V. hæmoroidalis*). It is, however, possible to find in some localities also females of *V. plumata* and *V. hæmoroidalis* which have a very reduced black thoracic spot of hairs. The black spot itself has often a brown color in the females, but never in the males. The *V. altaica* from Asia is extremely dimorphic in regard to this black spot. The male (Pl. I., Fig. 9) has a perfectly black thorax, the female has the usual black spot in relation to its size and coloration like the males of *V. hæmoroidalis* (Pl. I., Fig. 4) and *V. plumata* (Pl. I., Figs. 6, 7). The yellow spot of *V. Bombylans* var. *flava* is inherited. I had in one culture (unpublished data) 6 females in F_1 of *V. Bombylans* var. *flava* from a female *V. Bombylans* var. *flava* (Pl. I., Fig. 8).

The *V. Bombylans* var. *caucasica*, which lives in bumblebee nests of the Caucasian mountains, has a white thorax (the black spot is present in the males), and the upper part of the abdomen is also white, but the third to sixth abdominal rings are exactly the same as in the European *V. Bombylans* and *V. hæmoroidalis* (compare Figs. 1, 2, 3, 4, 8, Pl. I.).

On looking at this fly one gets the impression that only the yellow hairs of the European variety *V. plumata* and *V. hæmoroidalis* have changed into white, but that the black and fulvous hair covering the end of the abdomen remained unaffected. A parallel color change occurs in almost all black bumblebee species which inhabit the Caucasus. The *Volucella Caucasica* is thus similar to the Caucasian *B. eriophorus*, *B. niveatus* and other species of this region, which have white patches of hair on the thorax. The black and rufous parts of the bumblebees' abdominal rings, which correspond to the black and rufous bands of the endemic *Volucella* flies remained also unaffected and similar in coloration to those of the European *Bombus lapidarius*, *B. confusus* and *Volucella Bombylans* (type). Many other Caucasian flies (*Cheilosia œstracea*, *Tabanus gudaurensis*, *T. tricolor*, etc.) have similar color changes parallel to those described above. The appearance of white spots or bands is characteristic for both bumblebees and flies of this mountain region.

Summarizing these data we can say that there is a striking phenomenon of color convergence between European *Syrphidæ* (especially *Volucella*, and its varieties), *Tabanidæ*, *Asilidæ* and *Œstridæ* and bumblebees, which are distributed in the same geographical area. A similar parallelism in coloration can be also noticed for the Caucasian region. It must be also pointed out that there is a predominance of certain color patterns for the European group of bumblebee-like flies and bumblebees. The combination of black and rufous, white and yellow, and black and white stripes is more often found than black and yellow. This is a characteristic feature of both insect genera in the European part of the old world.

An entirely different situation is found in the United States of America. Even a superficial survey of the collections of American (Syrphidæ and Asilidæ) flies and bumblebees shows distinctly, that both insect genera have, strictly speaking, only one pattern, namely, black and yellow striped individuals. (Only two species, *B. occidentalis* and *B. borealis*, have parts of their body covered with white hairs.) The combination of black and rufous, yellow and white, black and white, is absolutely absent or very rare on this continent. The overwhelming numbers of black and yellow striped specimens among these flies and bumblebees is a peculiar feature throughout the United States. The phenomenon of color convergence has reached its climax in North America.

THE NORTH AMERICAN GROUP OF *Volucella Bombylans.*

The systematic relationship of the American varieties of *V. Bombylans* has been recently studied by Johnson ('16, '25) who divides these flies into two large groups. To the first (the black-faced group) belongs the eastern *V. evecta*, var. *V. evecta americana* var. *V. evecta sanguinea*, and the boreal *V. evecta arctica*. To the second (the yellow-faced group) belongs the western *V. facialis*, the Canadian *V. facialis lateralis* and the *Vol. facialis* var. *rufomaculata*, which is found in Colorado and along the Rocky Mountains. Excepting the coloration of the pleura, which may be yellow or black in both groups, and excepting differences in the color patterns of the thorax and abdomen, no other distinguishing characters have been detected. It is very probable that all European and American varieties might interbreed.

CONVERGENCE OF COLORATION BETWEEN FLIES OF THE EASTERN STATES AND BUMBLEBEES OF THE SAME REGION.

Volucella bombylans var. *evecta-americana* (PL. I., 15).

The eastern *Volucella bombylans* var. *evecta* (Pl. I., 15) *americana* is entirely pale yellow except the last two to four posterior abdominal segments, which are covered with black hairs. The same distribution of yellow and black hairs on the thorax and abdomen is characteristic for all the eastern species of pilose flies. *Mallota posticata* (Pl. I., 18, 19), *Mallota cimbiciformis* (Pl. I., 20–22), *Eristalis flavipes* (Pl. I., 23–24), *Eristalis bastardii* (Pl. I., 26–27), *Dasyllis lata* (Pl. II., 9), *Dasyllis marquarti* (Pl. II., 9), *Das. sacraton* (Pl. II., 9), *Das. champlaini* (Pl. II., 8). All these *Syrphidæ* and *Asilidæ* are very common in the eastern states and can be found on the same flowers with *Bombus*.

Six bumblebee species of the eastern states, namely *Bombus vagans* (Pl. III., 1), *B. affinis* (Pl. III., 7), *B. impatiens* (Pl. III., 3), *B. separatus* (Pl. III., 2), *B. bimaculatus* (Pl. III., 5), *B. perplexus* (Pl. IV., 4) have a coloration exactly corresponding to *Volucella evecta americana* (Pl. I., 15) and to the other *Syrphidæ* and *Asilidæ* which are mentioned above. (Compare Pl. I., 15, with Pl. III., 1, 7, 3, 2, 5, 4. Compare also Pl. II., 7, 8, 9, with Pl. III., 1, 7, 3, 2, 5, 4 and then Pl. II., 10, 11, with Pl. III., 13, 14, 15.)

Four other eastern bumblebee species, namely *B. pennsylvanicus* (Pl. IV., 13, 14), *B. auricomus* (Pl. IV., 13, 14), *B. terricola* (Pl. IV., 14) have a slightly different distribution of black and yellow hairs on the thorax and abdomen. This group of bees has its corresponding group of flies. It is hardly possible to detect any difference between the eastern *Eristalis flavipes* (Pl. I., 24) var. *b.*, *Dasyllis dithoracica* (Pl. II., 10, 11), *Das. grossa* (Pl. II., 11) and the bumblebees enumerated above. The flies are generally like the females, workers and males of the corresponding bumblebee species, but this is only true when the Hymenoptera has no polymorphic females or males. *B. bimaculatus* (Pl. III., 5) of this first eastern group, which is like *V. evecta americana*, has a male variety colored like *Eristalis flavipes* var. *b* (Pl. I., 24), *Eristalis flavipes*, on the other hand, has just been compared with the bumblebees of the second eastern group. (The groups are divided here only in relation to their color patterns.) We find the same phenomenon in *B. affinis* (Pl. III., 7), and *B. pennsylvanicus*, both of which have three types of males; (Pl. IV., 10, 11. 15–18) one of these is like the female, but the two others are entirely different. The latter two are extremely like *Criorhina verbosa* (Pl. II., 14) and *Criorhina Kinkaidi* var. *b* (Pl. II., 16).

As a rule, it is possible to maintain that most of the bumblebees' males are like the *Criorhina* flies. This is due to the fact that the males, like these flies, have reduced black bands which make them almost indistinguishable.

From the above we might infer that there is a very close resemblance between bumblebees and flies of the eastern parts of the United States. Yet it would be a mistake to conclude from this that there is mimicry between both genera; it must not be forgotten that there is also the same convergence in coloration, even as regards details, within the group of bumblebees and also within the group of flies or, more exactly, there is not only a correspondence between the flies and bumblebees of a given district, but the several species of Hymenoptera found living in a region resemble one another closely, and species of flies are also much alike among themselves. For example, when the bumblebees are compared with one another, we see that the coloration of the variety of *B. auricomus* (Pl. III., 13, 14), with the yellow scutellum, reappears in the same pattern in *B. pennsylvanicus* (Pl. III., 13, 14). One of the males of *B. bimaculatus* (Pl. III., 9), is similar in this respect to one of the males of *B. affinis* (Pl. III., 11). Many other coincidences are also evident and not uncommon in these groups. (Compare figures.) The coloration of the European *B. lapidarius* reappears in *B. confusus*, in many other species and in *Volucella Bombylans* (type) (Pl. I., 1).

The distribution of *V. b. evecta americana* is probably by far more restricted than that of the above mentioned bumblebees, which may be found further to the south. This can be explained through the fact that all large *Volucella* are circumboreal species and never appear farther to the south than the 42d parallel, unless there are mountains which extend to the south. Favorable conditions are thus produced and the bumblebee nests may be found again infested with *Volucella* larvæ in more elevated zones. *Volucella evecta americana* is not to be found much farther to the north. According to Johnson this variety can be collected along the Atlantic coast as far north as Mount Desert, but there this fly is already replaced by *Volucella bombylans facialis lateralis* (Pl. I., 11), which belongs to the Canadian zone.

Volucella bombylans evecta (Walker) (Pl. I., 14).

The thorax, scutellum and the three first abdominal rings of this fly correspond to *V. B. evecta americana*, (Pl. I., 15), but the posterior 4–6 segments may be either yellow or reddish. *Evecta* is the upper austral form, extending through the transition zone. This fly resembles closely the males of the eastern bumblebees. Its coloration has altogether the aspect of these Hymenoptera, but a strict parallelism in color patterns with any of the eastern bumblebees is not traceable. *Volucella evecta* is a rare form when compared with *V. evecta americana*. It is possible that a detailed study of its distribution in the east might explain its color patterns.

Volucella bombylans evecta sanguinea (Pl. I., 16).

This variety is an extremely rare variation of *V. B. evecta americana*. It has been recorded in the eastern states. This fly has yellow hairs on thorax, scutellum and abdomen, except the third abdominal ring, which is covered with fulvous pile. The sides of this segment have some black hairs. This *Volucella* represents, in a way, a parallel variation to that of *V. rufomaculata* in the subspecies *facialis*, which is distributed along the more elevated portions of the Rocky Mountains. *V. sanguinea* is not comparable to any bumblebee species of the eastern United States. A third and analogous parallel variation is found in *Eristalis flavipes* var. *melanostoma* (Pl. I., 25) of the east, and a fourth in *Dasyllis fernaldi* (Pl. II., 18) an Asilid fly of the Rocky Mountains.

The sporadic reappearance of parallel variations in *V. sanguinea* and *Eristalis flavipes*, which are analogous to the variety of *V. rufomaculata* and *Dasyllis fernaldi* (Pl. II., 18) of the west, suggests that the genic mutations producing these color changes in some individuals are probably of a related chemical structure, in the sense of their end effect. It is also interesting that they affect the same parts of the insect's body. The same argument can be applied to the bumblebees. It would be more difficult to explain, however, the way in which a new mutation could replace the more abundant form for a given geographical zone.

Convergence of Coloration between Flies of the Arctic Zone.

Volucella bombylans arctica (Pl. I., 17). This syrphid of the *evecta* group is entirely dark yellow, with a short and unfinished black band on the thorax. This *Volucella* is extremely like in coloration to the arctic bumblebee—*Bombus gelidus* (Pl. IV., 1), and *B. borealis* (not figured).

Convergence of Coloration between Flies of the Rocky Mountains and Bumblebees of this Region.

The Volucella facialis Group, Volucella Bombylans facialis var. rufomaculata (Pl. I., 13).

Volucella rufomaculata, which is a parallel variation to the *V. evecta sanguinea* of the *evecta* group is limited to the mountain region of Colorado, Utah and New Mexico. The dorsum of the thorax is black pilose-like in all varieties of the *facialis* group. This is a characteristic which is entirely absent in the *evecta* group (compare Pl. II., 14–17). In this respect the male and female flies of the *evecta* group are like the females of the European varieties of *Volucella Bombylans*, and the male and female flies of the *facialis* group can be compared to the males of the European varieties of *Volucella plumata* and *hæmoroidalis*. The pleura of the facialis flies are black as in the European varieties. It is not improbable that the western American *Volucella* group is more closely related to the European group.

It is again striking that *V. rufomaculata* has a coloration resembling that of most of the bumblebees of this mountain region. An exact coincidence in color patterns can be observed in *Bombus sylvicola* (Pl. IV., 5), *B. huntii* (Pl. IV., 1–2), *B. melanopygus* (Pl. IV., 4), *B. eduardii* (Pl. IV., 13–17), *B. ternarius* (Pl. IV., 6). The same is true for the Asilid fly *Dasyllis fernaldi* (Pl. II., 18).

Five other bumblebee species of the Colorado area, namely, *Bombus centralis* (Pl. IV., 18), *B. borealis* (Pl. IV., 12), *B. gelidus* (Pl. IV., 1), *B. flavifrons* (Pl. IV., 7, 8), *B. rufocinctus* (Pl. IV., 9), *B. kirbuellus* (not figured), *B. appositus* (Pl. IV., 12) have also the same type of coloration with the characteristic rufous abdominal segment like that of *V. rufomaculata*. The coincidence in coloration of the segments is not so exact as in the first four bumblebee species enumerated above, but the reappearance of some segments with red pile must be regarded as a characteristic feature in both insect genera of this region. It is strange that the Caucasian mountain species of flies and bees are partly changing their pile into a shining white color, whereas the American mountain species tend to turn into a bright vermilion coloration.

Another group, consisting of bumblebees with a shining wax yellow coloration is also present in the Colorado region. This includes *B. sonorus* (Pl. IV., 3), *B. morissoni* (Pl. IV., 4), *B. nevadensis* (Pl. IV., 5), *B. terricola* (Pl. III., 14). As regards the distribution of black and yellow bands, the first three species are like the first group of the eastern bumblebees, and the fourth one is like the bumblebees of the second eastern group. The main difference is observed in the tint of the yellow pile, which is extremely bright in *B. morissoni*, *nevadensis*, *sonorus* and *terricola*. It is apparently again a case of color convergence, but which is present only in the hymenoptera group, as no flies of this kind have been collected in this mountain zone. Occasionally it is possible to find *Volucella* flies with the more brilliant wax yellow pile, but it must be pointed out that the yellow hairs of the flies vary in intensity of pattern from light ochre to swarthy brown, or more exactly from maize yellow through wax yellow to deep colonial buff. Many slight variations can also be noticed in the red pile. The black color on the contrary is always constant. Many Asilidæ are like these bumblebees; an exact distribution of the same colored bands can be observed in *Dasyllis lata* var. *a. D. champlaini*, *D. macquarti*, *D. grossa* ♀ (Pl. II., 7, 8, 9, 12). *Mallota posticata* (Pl. I., 19).

Convergence of Coloration between Flies of the Pacific Coast and the *Bombus* Species which Inhabit the Same Region.

The Volucella bombylans facialis (Pl. I., 12).

This fly is one of the darkest when compared with the other pilose species of this family. The pleura are black pilose; the dorsum of the thorax has a larger area covered with black hairs than in all other *Volucella* flies. The black hairs of the third abdominal segment extend also partly onto the second ring, and sometimes on to the upper quarter of the fourth abdominal ring. The last segments are yellow pilose or reddish. An increase of black hairs on thorax, scutellum and abdomen is also characteristic for the endemic bumblebees. *Bombus vosnesensky* (Pl. IV., 6), *B. Crotskii* (Pl. IV., 7), *B. californicus* (Pl. IV., 8–11) are good examples of this. *Volucella facialis* is distributed along the Pacific coast and in Alaska. The coincidence in the distribution of black and yellow bands in *Volucella* flies and bumblebees of this zone is not absolutely exact, but yet the increase of the black hairs and the distinct and parallel process of melanization in both insect genera is strikingly evident.

Volucella B. facialis lateralis (Pl. I., 11).

This fly is extremely like the former one, but the pleura are yellow pilose, and the black spot on the dorsum of the thorax has a more reduced area than in *V. facialis*. *V. lateralis* belongs to the Canadian zone (Alaska to Newfoundland, Mt. Desert) and is, so to speak, the eastern representative of *facialis*. I have no data as to other flies and bumblebees of this large area, and a comparison has thus been impossible.

SUMMARY.

1. The European Syrphid fly *Volucella Bombylans* and its varieties, *V. hæmoroidalis* and *V. plumata* have a coloration which corresponds to the coloration of the majority of European bumblebees. The same is true of many other pilose flies of Europe.

2. The Caucasian *Volucella bombylans caucasica* has a coloration which corresponds to the coloration of various bumblebees of this mountain region. The same is true for other pilose flies of this zone.

3. The American *Volucella bombylans evecta-americana* of the eastern states and 8 other species of Syrphid flies have exactly the same color patterns as six different *Bombus* species of this region; four different *Bombus* of the same area have the same type of coloration as three other beelike flies of the eastern states.

4. *Volucella bombylans evecta sanguinea*, and *Eristalis flavipes* var. *melanostoma* of the eastern states are rare and parallel

variations which have analogous coloration to that of *Volucella bomb.* var. *rufomaculata* of the facialis group and of the Rocky Mountain region.

5. *Volucella bombylans arctica* of the *evecta* group is similar in coloration to the arctic bumblebee *Bombus gelidus* and to other species.

6. *Volucella bombylans* var. *rufomaculata* of the Rocky mountains has a similar coloration to twelve different bumblebee species which occur in the same region. The Asilid fly *Dasyllis fernaldi* of the same zone has a corresponding coloration to *V. rufomaculata* and to the twelve bumblebee species.

7. *Volucella bomb. facialis* of the Pacific coast has analogous color patterns to three bumblebee species of this zone. *Volucella bomb. facialis lateralis* of the Canadian zone and *V. bomb. evecta* of the eastern states have not been studied on account of absence of data as to their distribution.

I am greatly indebted to Dr. A. Sturtevant, Dr. Johnson and Dr. Lutz, for the use of their collections of flies and of *Bombus*, also for their help and advice in this work.

LITERATURE.

C. W. Johnson.

'16 The *Volucella Bombylans* group in America. Psyche, Vol. XXIII., No. 6.

'25 The North American Varieties of *Volucella bombylans.* Psyche, Vol. XXII., April 1925.

E. Gabritschevsky.

'24 Farbenpolymorphismus und Vererbung mimeticher varietäten der Fliege *V. Bombylans* und anderes Zweiflügler. Zeitschr. für Induktive Abst. und Vererbungslehre, Bd. XXXII., Vol. 1, 2.

C. E. Keeler.

'26 Recent work by Gabritschevsky on the Inheritance of Color Varieties in *Volucella Bombylans.* Psyche (A Journal of Entomology), Vol. XXXIII., No. 1, February 1926.

EXPLANATION OF PLATES I–IV.

All figures represent flies in a schematized way. The upper square is the thorax; the second one is the scutellum; the third is the abdomen. The dotted line between the abdomens of different flies shows the segments of the insects. The head is not drawn. In Plate I. the black color corresponds to the black pile, the white to white hairs, the spotted parts are of a yellow coloration, and the vertical parallel lines correspond to a cadmium orange tint in the flies. In all the other plates, (I. (11–27) and III., IV.) the uncolored parts of the schemes correspond to different tints of yellow (the exact color stands in the explanation of the plates; these colors have been compared with Ridgway's Color Standards—R. Ridgway, "Color

Standards and Color Nomenclature," 1912, Washington). The black parts are again black in the flies and bumblebees; the striped individuals have on those segments a cadmium orange coloration. The exact shades of these different red tints are given under every figure in the explanation of the plate.

Explanation of Plate I (Nos. 1–10).

The European and Asiatic Groups of Volucella Bombylans.

1. *Volucella Bombylans*, ♂, ♀.
2. *Volucella Bombylans*, var. *rufa* ♀.
3. *Volucella Bombylans*, var. *flava*. ♀.
4. *Volucella B. hæmoroidalis* ♂, and *Volucella B. altaica* ♀.
5. *Volucella B. hæmoroidalis* ♀.
6. *Volucella B. plumata* ♂.
7. *Volucella B. plumata* ♀.
8. *Volucella caucasica* ♂.
9. *Volucella altaica* ♂.
10. *Volucella plumata* var. *a* ♀.

The yellow pile is variable. Specimens of these flies are found with a maize yellow (19–40, *y. f.*, Pl. IV.), wax yellow (21, *o–yy* Pl. XVI.), and deep colonial buff yellow (21, 0, Pl. XXX.). The red pile is less variable, yet specimens with a cadmium orange (13, 0–0, Pl. III.), or Mars yellow (15–00, Pl. III.), or ochraceus tawny (15–00, Pl. XV.) are found.

The white pile is snow white, or with a slight yellow tint.

Explanation of Plate I. (Nos. 11–27).

The North American Group of Volucella bombylans.

11. *Volucella bombylans facialis lateralis*, ♂, ♀.	The *facialis* group—maize yellow.
12. *V. B. facialis*, ♂, ♀.	
13. *V. B. facialis* var. *rufomaculata*, ♂, ♀.	
14. *V. B. evecta*, ♂, ♀.	The *evecta* group—maize yellow.
15. *V. B. evecta-americana*, ♂, ♀.	
16. *V. B. evecta* var. *sanguinea*, ♀.	
17. *V. B. evecta artica*, ♂, ♀.	

The North American Bumblebee-like Syphidæ 11–27.

The yellow pile is maize yellow (19–40, *y. f.*, Pl. IV.), the red pile is cadmium orange (130–0 Pl. III.).

18. *Mallota posticata*, ♂, ♀.	Wax yellow or maize yellow.
19. *Mallota posticata* var. *a*, ♂, ♀.	
20. *M. cimbiciformis* var. *a*, ♂, ♀.	Gray pile.
21. *M. cimbiciformis* var. *b*, ♂, ♀.	Wax yellow pile.
22. *M. cimbiciformis* var. *c*, ♂, ♀.	
23. *Eristalis flavipes* var. *a*, ♂, ♀.	Wax yellow or maize yellow.
24. *Er. flavipes* var. *b*, ♂, ♀.	
25. *Er. flavipes* var. *melanostoma*, ♀.	
26. *Er. bastardii*, ♀.	Maize yellow.
27. *Er. bastardii*, ♂, ♀.	

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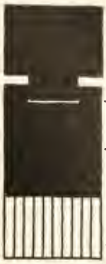

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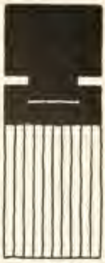

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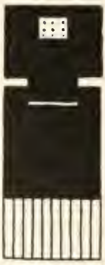

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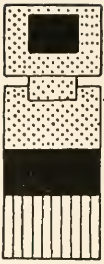

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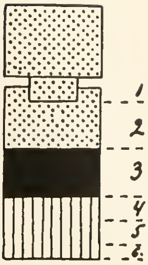

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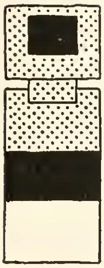

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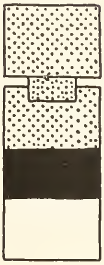

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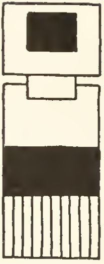

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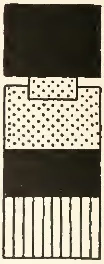

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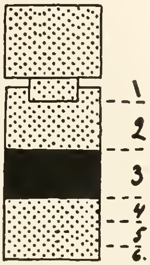

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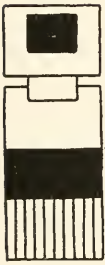

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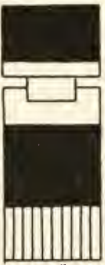

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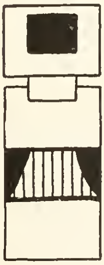

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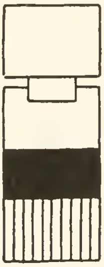

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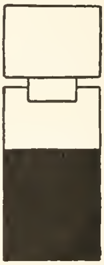

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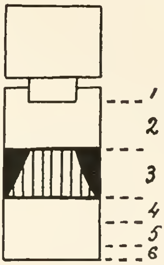

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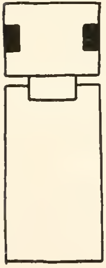

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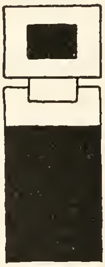

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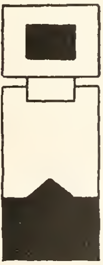

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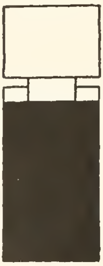

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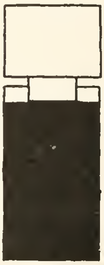

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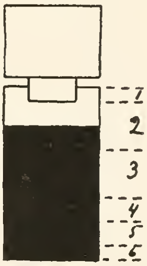

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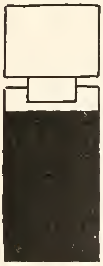

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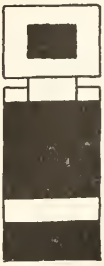

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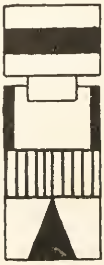

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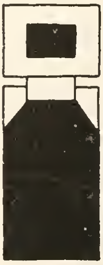

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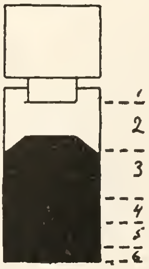

Explanation of Plate II.

The North American Bumblebee-like Asilidæ, and Flies of the Criorhina Group.

1. *Dasylus unicolor*, ♂, ♀.
2. *Dasylus insignis*, ♂, ♀.
3. *D. posticata*, ♂, ♀.
4. *D. divisor*, *D. cinerea*, ♂, ♀.
5. *D.* sp. ?, ♂, ♀.
6. *D. flavicola*, ♂, ♀; *D. virginica*, ♂, ♀; *D. grossa*, ♂, ♀.
7. *D. lata* var. *a*, ♂, ♀. } Wax yellow of deep colonial buff.
8. *D. champlaini*, ♂, ♀.
9. *D. lata* var. *b*, ♂, ♀; *D. marquarti*, ♂, ♀; *D. sacraton*, ♂, ♀.
10. *D. dithoracica*, var. *a*, ♂, ♀.
11. *D. dithoracica* var. *b*, ♂, ♀; *D. grossa*, ♀.
12. *D. grossa* var. *a*, ♀.
13. *Criorhina nigripes*, ♂, ♀.
14. *Criorhina verbosa*, ♂, ♀.
15. *Criorhina kincaidi* var. *a*, ♂, ♀. } Deep colonial buff.
16. *Criorhina kincaidi* var. *b*, ♂, ♀.
17. *Cephenomya abdominalis*, ♂, ♀.
18. *Dasylus fernaldi*, ♂, ♀. } Cadmium orange, maize yellow.

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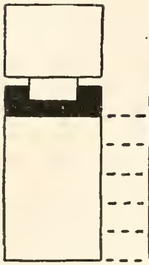

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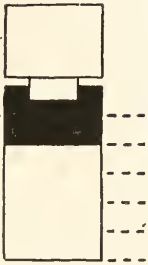

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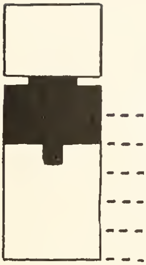

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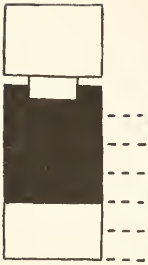

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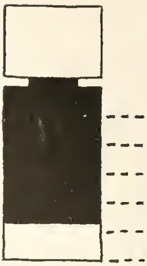

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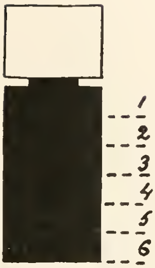

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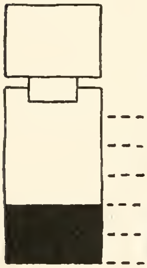

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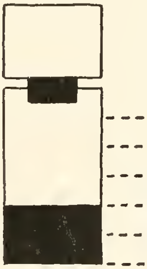

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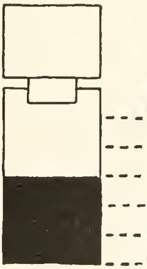

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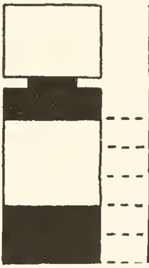

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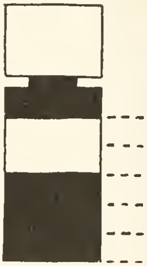

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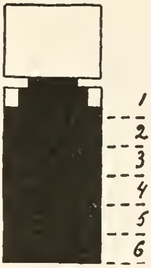

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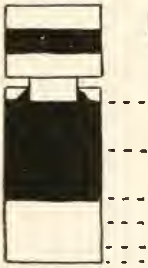

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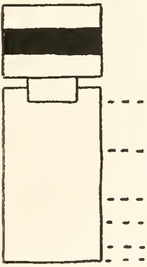

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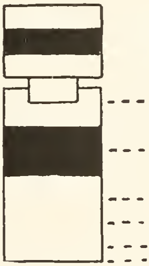

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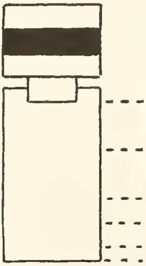

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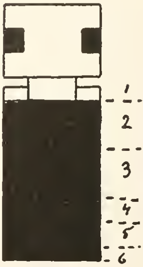

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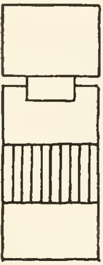


E. GABRITSCHEVSKY.

19

EXPLANATION OF PLATE III.

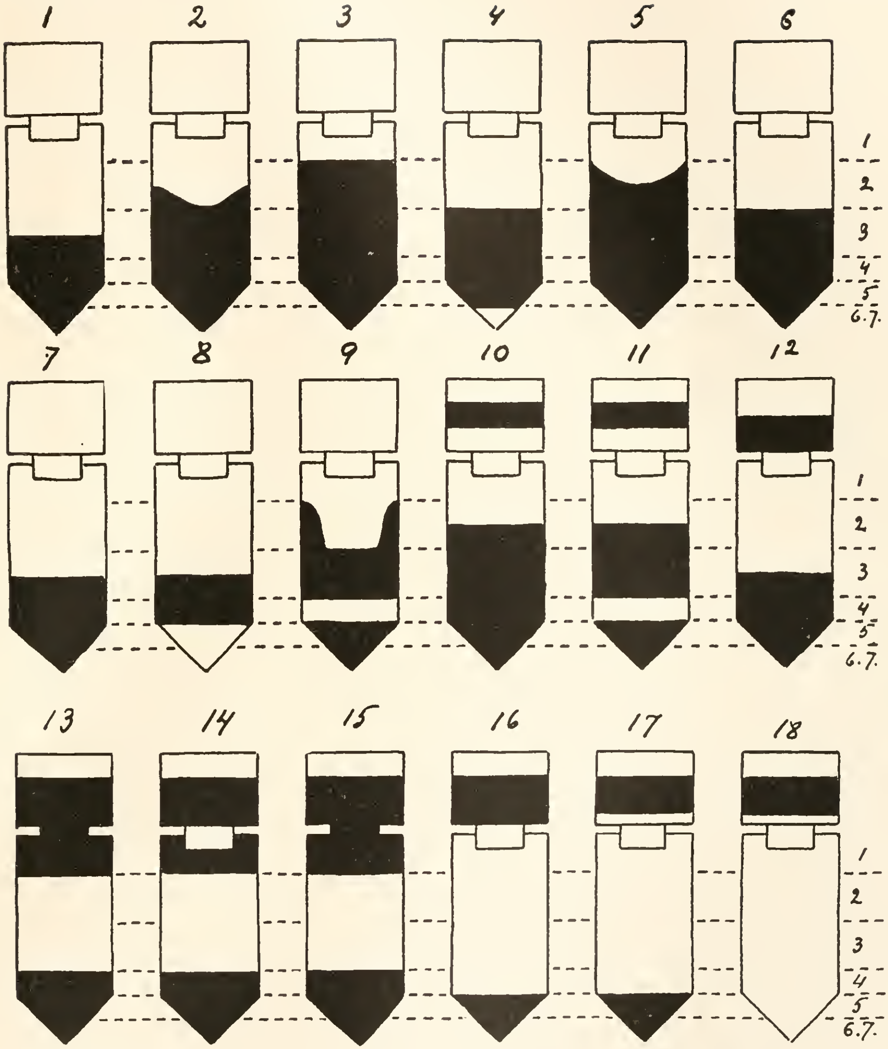
Bumblebees of the Eastern States of North America.

First Group 1–11.

1. *Bombus vagans*, ♀, ☿, ♂. (Deep colonial buff 21″, *o*—Pl. XXX.)
2. *B. seperatus*, ♀, ☿, ♂. (Deep colonial buff—Pl. XXX.)
3. *B. impatiens*, ♀, ☿, ♂. (Deep colonial buff—Pl. XXX.)
4. *B. perplexus*, ♀, ☿, ♂. (Deep buff yellow—Pl. IV.)
5. *B. bimaculatus*, ♀, ☿, ♂. (Deep colonial buff, 21″, *o*—Pl. XXX.)
6. *B. bimaculatus*, ♀, ☿, ♂. (Deep colonial buff—Pl. XXX.)
7. *B. affinis*, ♀, ☿, ♂. (Deep colonial buff—Pl. XXX.)
8. *B. vagans*, —, —, ♂. (Deep colonial buff—Pl. XXX.)
9. *B. bimaculatus*, —, —, ♂. (Deep colonial buff—Pl. XXX.)
10. *B. affinis*, —, —, ♂. (Deep colonial buff—Pl. XXX.)
11. *B. affinis*, —, —, ♂. (Olive buff, 21″, *o*—Pl. XL.)

Second Group 12–18.

12. *B. fraternus*, ♀, ☿, ♂. (Deep colonial buff 21″, *o*—Pl. XXX.)
13, 14. *B. pensylvanicus*, ♀, ♀. (Mustard yellow 19′, *b*—Pl. XVI.)
13, 14. *B. auricomus*, ♀, ♀. (Deep colonial buff, 21″—Pl. XXX.)
14. *B. terricola*, ♀, ☿, ♂. (Apricot yellow—Pl. IV.)
15. *B. pensylvanicus*, ♂. (Mustard yellow, 19′—Pl. XVI.)
16. *B. pensylvanicus*, ♂. (Mustard yellow, 19′—Pl. XVI.)
17. *B. pensylvanicus*, ♂. (Pyrite yellow, 23′—Pl. IV.)
    B. fervidus, ♀, ☿, ♂.
18. *B. pensylvanicus*, ♂. (Pyrite yellow, 23′—Pl. IV.)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Explanation of Plate IV.

Bumblebees of the Rocky Mountains.

1. *B. huntii*, ♀, ☿, ♂. (Olive ochre, 21″—Pl. XXX.)
2. *B. huntii*, —, —, ♂. (Amber yellow, 21″—Pl. XVI.)
   (3d and 4th abd. segments cadmium orange, 130—Pl. III.)
3. *B. melanopygus*, ♀. (Cartridge buff 19″—Pl. XXX.)
   (3d and 4th abd. segments cadmium orange 130—Pl. III.)
4. *B. melanopygus*, ♂. (Segments cadmium orange—Pl. III.)
5. *B. sylvicola*, ♀, ☿, ♂. (Straw yellow, 21′—Pl. XVI.)
   (3d and 4th abd. segments orange rufous, 11′, *i*—Pl. II.)
6. *B. ternarius*, ♀, ☿, ♂. (Straw yellow, 21′—Pl. XVI.)
   (3d, 4th and 5th abd. segments cadmium orange—Pl. III.)
   (130—light buff, 17′—Pl. XV.)
7. *B. flavifrons*, ♀, ☿. (Thorax light buff, 17′, *o*—Pl. XV.)
   (Abdomen ochraceus tawny, 15—Pl. XV.)
8. *B. flavifrons*, ♂. (Antimony yellow, 17′, *b*—Pl. XV.)
9. *B. rufocinctus*, ♀, ☿. (Thorax, colonial buff, abdomen olive ochre, 21″—Pl. XXX.)
10. *B. rufocinctus*, ♂. (Colonial buff.)
11. *B. rufocinctus*, ♂. (Colonial buff.)
12. *B. apositus*, ♀, ☿, ♂. (Thorax white with gray.)
    B. borealis, ♀.
13. *B. edwardsii*, ♀, ☿. (Maize yellow, 19—Pl. IV.)

14–15. *B. edwardsii*, ♂. (Maize yellow—Pl. IV.)

16. *B. edwardsii*, ♂. (Cadmium orange, 13—Pl. III.)
17. *B. edwardsii*, ♂. (Cadmium orange, 13—Pl. III.)
18. *B. centralis*, ♀, ☿. (Maize yellow, 19—Pl. —.)
    (Cadmium orange, 13—Pl. II.)

Explanation of Plate IV.

The Arctic Species of Bumblebees (Labrador).

1. *B. gelidus*, ♀, ☿.
2. *B. frigidus*, ♀, ☿.

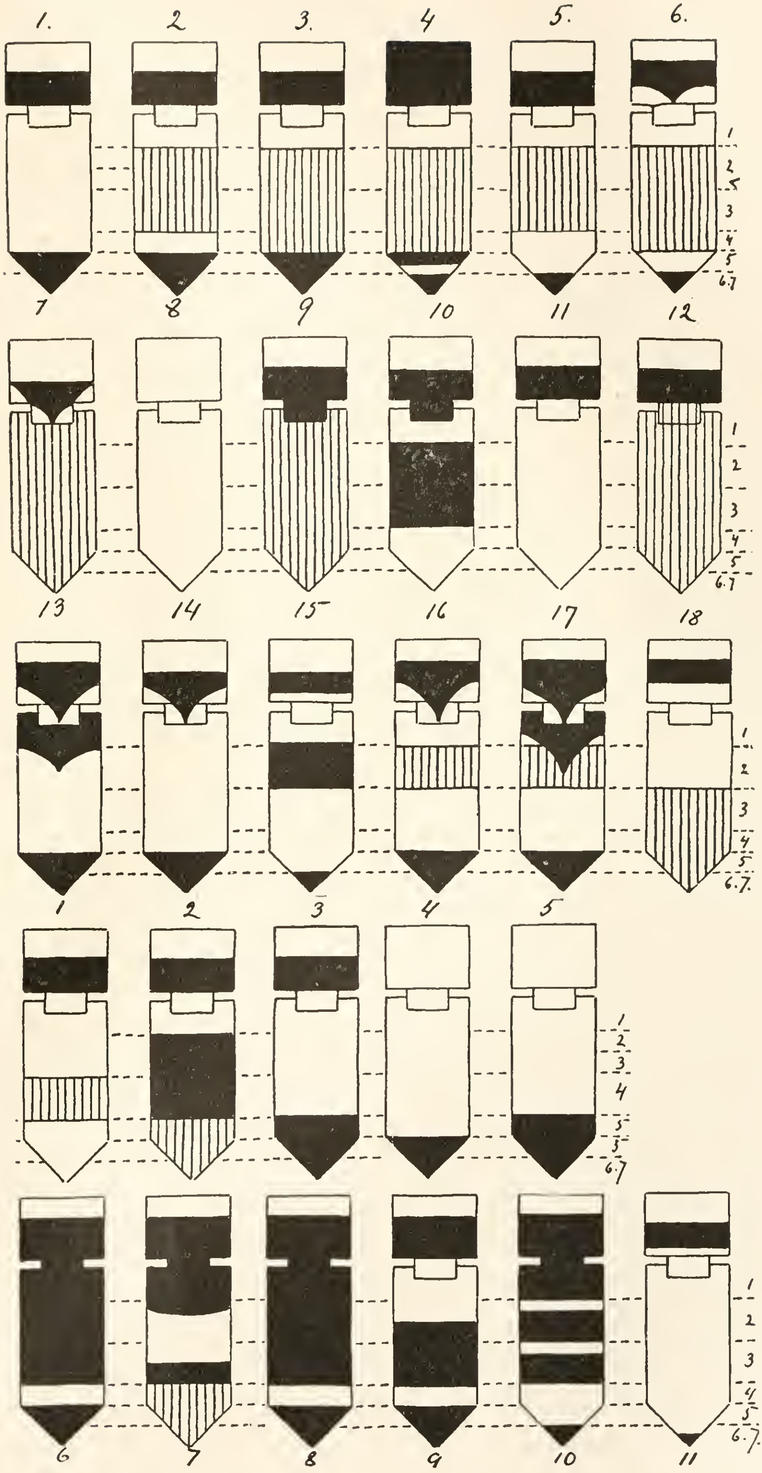
The Wax Yellow Group of Rocky Mountain Bumblebees.

3. *B. sonorus*, ♀, ☿. (Wax yellow, 21′—Pl. XVI.)
4. *B. morrissoni*, ♀, ☿. (Wax yellow—Pl. XVI.)
5. *B. nevadensis*, ♀, ☿. (Antimony yellow, 17′—Pl. XV.)

Explanation of Plate IV.

Bumblebees of the Pacific Coast (California-Alaska).

6. *B. voznosenskii*, ♀, ☿, ♂. (Pinard yellow, 21′—Pl. IV.)
7. *B. crotshii*, ♀, ☿, ♂. (Deep colonial buff, 21′—Pl. XXX.)
   (4th, 7th segments flame scarlet—Pl. II.)
8. *B. californicus*, ♀. (Chartreuse yellow, 25″—Pl. XXX.)
9. *B. californicus*, ♀. (Chartreuse yellow—Pl. XXX.)
10. *B. californicus*, ♂. (Yellow ochre, 17′—Pl. XV.)
11. *B. californicus*, ♂. (Yellow ochre—Pl. XXX.)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Vol. LI. *November, 1926.* *No. 5.*

BIOLOGICAL BULLETIN

THE FLAGELLATE FAUNA OF THE CŒCUM OF THE STRIPED GROUND SQUIRREL, *CITELLUS TRIDECEMLINEATUS*, WITH SPECIAL REFERENCE TO *CHILOMASTIX MAGNA* SP. NOV.

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IOWA STATE COLLEGE.

The intestinal protozoan fauna of the rat, mouse, guinea pig and rabbit has been investigated quite extensively. Not so much has been done on field rodents as on those which lend themselves to laboratory use. In the autumn of 1925 and spring of 1926 the writer made a study of the protozoa harbored in the alimentary tract of the striped ground squirrel, *Citellus tridecemlineatus*. This rodent was found to be quite as rich a field for investigation of protozoan parasites as the aforementioned animals.

The protozoa so far found to be residents of the cœcum of the striped ground squirrel comprise one species of amœba, *Endamœba citelli;* one species of *Chilomastix;* two species of *Trichomonas*, one of them probably new; one species of *Tetratrichomastix*, subgenus of *Eutrichomastix;* one species of *Hexamitus;* and a flagellate which is either a species of *Hexamitus* or *Urophagus*. In the small intestine a *Giardia* was found. *Endamœba citelli*, and its pathogenic parasite, *Sphærita endamœbæ*, were described by the writer (1926) in a previous paper. The *Giardia* was sent for identification to Dr. R. W. Hegner, who found it to be a new species.* The *Chilomastix*, *Trichomonas*, *Tetratrichomastix*, and *Hexamitus* will be reported upon in this paper in the order named. The preparations of the other flagellate were not entirely suitable

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*Since this paper went to press a paper by Dr. R. W. Hegner has appeared in which this *Giardia* is described. See Hegner, R. W. 1926. *Giardia beckeri* n. sp from the ground squirrel and *Endamœba dipodomysi* n sp. from the kangaroo rat Jour. Paras. 12: 203–206.

for critical study, and the specimens were very scarce. Consequently a description of this species (*Hexamitus* or *Urophagus*) must be delayed until more suitable material is obtained. Twenty ground squirrels in all were examined for protozoa. All save one harbored infinite numbers of protozoa in the cœcum. This one had only a light infection.

The technique consisted of a careful microscopic examination of thin emulsions of the cœcal contents in normal salt solution. Permanent prepared slides were made by Heidenhain's iron-hæmatoxylin staining after fixation in Schaudinn's solution.

Chilomastix magna sp. nov. This protozoan was found in all twenty ground squirrels. The shape is essentially like that of *Chilomastix mesnili* from man, pyriform with three flagella at the broadly rounded anterior end. There is often a tuft of short bristly cuticular projections in the region of the flagella (Fig. 1). It may be present in either living or stained material, and is not a fixation artefact. Fixed and stained individuals measured from 10 to 21.6 micra in length and from 6 to 11 micra in width, the average width being about 9 micra. The length of *Chilomastix mesnili*, as given by Kofoid and Swezy (1920) is from 9.6 to 15 micra in stained preparations. The writer measured a number of *Chilomastix mesnili* on slides which he had prepared, and found a range of from 7 to 11 micra. When one considers that twenty out of thirty specimens of *Chilomastix magna* measured from 15 to 21.6 micra, it will be seen that this flagellate is almost twice as long as the one found in the intestine of man.

A study of the cytology of this protozoan promised to be particularly interesting because of the discrepancies between the accounts of *Chilomastix mesnili* by Kofoid and Swezy (1920) and Dobell and O'Connor (1921). The latter authors are particularly outspoken in flatly denying the existence of certain cell structures which the former claim to have seen. Without desiring to become in any way involved in the controversies of these writers, the writer must state that his observations agree more nearly with those of Kofoid and Swezy, although his interpretations may differ in certain respects. The morphology of the free form, cyst, and dividing individuals will be described.

The nucleus of the free form lies in the extreme anterior end of the body. Measurements indicate a diameter of from 3 to 4.5 micra. Anterior to the nucleus lie a number of deeply staining granules, concerning the affinities of which there is so much controversy. Kofoid and Swezy's account of the arrangement of these granules based largely upon their observations of the cyst is somewhat as follows. There lies upon the nuclear membrane a centrosome, which is connected by the "nuclear rhizoplast" with a "primary blepharoplast." This blepharoplast is joined to the "secondary blepharoplast" by means of the "transverse rhizoplast.". Finally, another thin thread-like line connects the secondary and tertiary blepharoplast. Directed somewhat posteriorly from the secondary and tertiary rhizoplast are two deeply staining fibrils, the one (parastyle) supporting the left lip of the cytostome, the other (parabasal body) supporting the right lip. Two of the three anterior flagella take their origin in the primary blepharoplast, one from the secondary. From the tertiary rhizoplast there arises, in addition to the "parabasal body," the cytostomal flagellum and a thread enclosing the mouth, the "peristomal fiber." The cytostomal flagellum vibrates along the edge of an undulating membrane. Dobell and O'Connor deny the existence of any integrated fibrillar system (neuromotor system), or connections between the granules at the anterior end, claiming in addition that there are six granules instead of four. Furthermore, in regard to the peristomal fiber they state, "We believe there is no such fibre, and that the mouth is not situated in this position."

Despite long and painstaking study the writer was not able to make out all the details of the granule complex with the positiveness of Kofoid and Swezy. That there is an integrated fibrillar system connecting the granules, however, the writer has no doubt. The rhizoplast connecting the blepharoplasts designated by Kofoid as secondary and tertiary is especially prominent in most specimens because of its thickness and deeply-staining qualities (Fig. 12). A fine rhizoplast running from a granule on the nuclear membrane was occasionally recognized. The best evidence for the fibrillar system, however, comes from the dividing stages, which will be described below. The writer was not able to make out the exact affinities of the flagella, because of the impossibility of resolving the granules in the granule complex. Fig. 12 represents one of the best observations which the writer was able to make.

A structure was noted which Dobell and O'Connor neither mention nor figure, but which resembled very much Kofoid and Swezy's figures of the peristomal fibril enclosing a more deeply-shaded granular area, the cytostome. In *Chilomastix magna*, this structure takes its origin in the blepharoplast complex, and passes posteriorly just dorsal to the cytostome and right supporting fibril (Kofoid's "parabasal body"), if we consider the cytostome to be on the ventral side. It usually extends far beyond the posterior curvature of this fibril into the posterior region of the body (Fig. 1). It appears to be surrounded by a fibril. This structure, however, has three dimensions, and is from one third to one half as deep as broad, as proved by polar views of a number of flagellates standing on end. So what appears to be a fibril, is in reality a membrane marking the boundary between this structure and the remaining cytoplasm. Rarely this structure appears to adhere to the right supporting fibril (Fig. 2). In others, no connection between the two is evident (Fig. 1). There is no connection between this body and the cytostomal flagellum, which lies between the right and left supporting fibrils (Fig. 1, 2, 12).

What is the function of this organelle? Is it an oral structure associated with the mechanism for the intake of food? It is not visible in the unstained living specimens, so this point could not be determined. Is it a reserve food supply? Failure to stain it either with iodine or *intra vitam* with Janus green gives no clue. For the present the writer prefers to call it a "parabasal body," because of its non-committal significance of anything as to function, although it does not stain with Janus green, which Shipley (1916) found would color the parabasal body of *Trypanosoma lewisi* and Becker (1923) found would color the parabasal bodies of *Crithidia* and *Herpetomonas*, suggesting their mitochondrial nature. Besides this structure, no other organelle resembling a peristomal fiber was seen. Might it not be that this body is much shorter in *Chilomastix mesnili*, and that the sharpness of its limiting membrane led Kofoid and Swezy to call it a peristomal fiber?

The cyst is typically lemon-shaped (Fig. 6). The size is markedly constant, every mature cyst measuring almost exactly 10 by 7.5 micra except one, which measured 12 by 8 micra. By way of comparison the writer measured twelve cysts of *Chilomastix mesnili*. These measured from 6 by 5 micra up to 8 by 7 micra. This indicates that the cyst of *Chilomastix magna* is somewhat larger than that of *Chilomastix mesnili*, but there is not the difference in size that there is in the free stages. The cyst contains a nucleus, the cytostomal structures, and the "parabasal body."

There is nowhere in the literature anything like a complete account of the cell division of *Chilomastix*. For this reason it was thought to be quite worth while to spend a large amount of time searching for dividing specimens, although they were extremely rare. As a result a fairly complete account of the process can now be given, although not all points have been cleared up.

What the writer conceives to be the resting nucleus is represented in Figs. 1, 7, and 12. There may be one blob of chromatin on the inner anterior surface of the nuclear membrane, or there may be one blob here and another in the posterior portion of the nucleus (Figs. 1, 12), or there may be a large number of smaller granules lying on the nuclear membrane (Fig. 7). There is a cloud of chromatin granules scattered thruout the entire nucleus. Very often at this time a small deeply staining spot, an endobasal body, or intranuclear kinetic element is visible (Figs. 7, 12).

A little later the granules become more concentrated and form a large endosome (Figs. 2, 9, 10). Careful study of this endosome shows that it is sometimes a concentration of granules (Fig. 2) and sometimes apparently a "chromatin knot" (Figs. 9, 10). Often a deeply-staining intradesmose is visible at the points where it passes from the endosome to the blobs of chromatin at one or both poles of the nucleus.

The endosomal chromatin becomes resolved into chromosomes, which appear to be dumbbell-shaped. It was not possible to count them exactly in any case, but the number is near ten. Strangely enough, the intradesmose could not be observed at this stage. Perhaps it lay too close to the nuclear membrane, for which there is some evidence to be given later.

The chromosomes arrange themselves in an equatorial plate, and a spindle forms. Fig. 11 is an exact drawing of the spindle at the time it first comes into evidence. The poles of the spindle are the blobs of chromatin at the poles of the nucleus in which the intradesmose had previously terminated. There is some evidence that these blobs of chromatin are not the centrioles, but that the centriole lies within them; *e.g.*, Fig. 2 shows what appear to be two centrioles lying against the chromatin blobs.

As mitosis progresses the spindle elongates considerably, the nucleus elongating simultaneously and the nuclear membrane commencing to dissolve. The polar blobs of chromatin disappear, leaving the tiny centrioles at the poles of the spindle (Fig. 3). The chromosomes of the dividing equatorial plate of the early anaphase become massed together so that it is not possible to count them, although in some cases they may appear as a mass of discrete granules.

The oral apparatus of the parent cell (right and left supporting fibrils, blepharoplasts, etc.) has degenerated. The centrioles divide, leaving connecting rhizoplasts between each centriole and its daughter blepharoplast. The daughter blepharoplast divides at least once (perhaps more), and from the products of the division the left and right supporting fibrils of the mouth grow out. The intradesmose is in evidence along the nuclear membrane, connecting the centrosomes at the poles of the spindle.

The intrazonal spindle fibers disappear in the late anaphase, and the two halves of the equatorial plate are disconnected from one another (Fig. 4). The remains of the spindle attaches the chromatin mass to the centrioles, while a fine rhizoplast connects the centriole with the blepharoplast complex. Fig. 4 appears inconsistent with Fig. 3 in regard to the points of attachment of the intradesmose which traverses the cell. It is extremely difficult to resolve the granules exactly, and the writer has put the intradesmose in each case exactly where it appeared to be. At this stage the "parabasal body" has appeared, and lies in its permanent position, just dorsal to the right supporting fiber.

The cell constricts in the middle (Fig. 5). The two daughter cells then reconstruct themselves, each with a complete new set of cell organelles. During division no food particles can be seen in the cell protoplasm.

Trichomonas muris (Hartmann) var. *citelli.* This flagellate was encountered in only three out of twenty ground squirrels. It is a common inhabitant of the cœcum of albino, house, and wild mice. The form from the ground squirrel, however, so closely resembles in its important features Wenrich's (1924) description of *T. muris* that it is considered to be at least a variety of this species. Furthermore, the chromosome count agrees with the number which Wenrich found in *T. muris.* The size of *T. muris* on prepared slides, according to Wenrich, ranges from 8 to 22 micra, with an average of 12.9 micra. The average of the body length of 100 individuals from the ground squirrel, not including the protruding axostyle, was 17 micra, somewhat larger than Wenrich's measurements.

The reader is referred to Wenrich's excellent account for the detailed description of this species. A rhizoplast connecting the nucleus and blepharoplast was seen by the writer in some cases. Andrews (1925) also saw it in *T. termopsidis.* One other rather unimportant respect in which the form from the ground squirrel differs from Wenrich's description is in the arrangement of the "inner row of chromatic granules." In the form studied by the writer the granules in this area constitute a field rather than a linear series (Fig. 13). In some specimens a small granular body was noted near the anterior end, although Schaudinn's fixative was used (Fig. 13). This was interpreted to be the parabasal body, as described by Wenrich. What the writer saw was evidently a residue left after the mitochondrial substance had been dissolved out. This parabasal body, however, does not stain *intra vitam* with Janus green.

Large numbers of dividing individuals were found. The writer has nothing to add to Wenrich's account. Several good chromosomes counts were made at the anaphase (Fig. 14) and prophase (Fig. 15). The number was six, in agreement with Wenrich. There seemed to be in most cases a fairly definite size range for the prophase chromosomes from one large chromosome down to a very small one (Fig. 15). Multiple mitosis or somatella formation of the nature of that observed and described by Kofoid (1915) was not seen in this material.

Trichomonas sp.? This small flagellate was present usually in large numbers in all ground squirrels examined. The body is elliptical, with a pointed posterior end, due to the protruding axostyle (Fig. 19). Length is from 6.5 to 10 micra without the axostyle which protrudes from 2 to 3 micra beyond the body. The nucleus lies at the anterior end. In the resting stage there is a round central chromatic endosome, an achromatic nuclear membrane, with a space between the endosome and membrane which is clear except for a sprinkling of fine granules. The endosome is prominently visible even in living unstained specimens. A large deeply staining basal body lies anterior to the nucleus. From it arise an axostyle which curves about the nucleus, and passes to the posterior end of the body; three anterior flagella; and a fourth flagellum which is directed toward the posterior. It lies in close contact with the body for about half the length of the cell, and the remaining length trails behind.

Typically, members of the genus *Trichomonas* possess an undulating membrane with a chromatic basal rod; *e.g.*, *Trichomonas muris*. This flagellate possesses no true undulating membrane, since the posterior flagellum which is the homologue of the marginal flagellum lies close to the body. It vibrates only slightly in the anterior region, while the free posterior end is quite active. Stained preparations show no chromatic basal rod. Whether these differences are sufficient to create a new genus is doubtful. Stained preparations often show a large open cytostome (Fig. 17). In living preparations it is usually closed.

Very few dividing forms were found, and hence no chromosome counts were made. Sometimes this flagellate is parasitized by a Sphærita (Fig. 19).

Tetratrichomastix citelli sp. nov. This flagellate (Figs. 16, 18) was present in nineteen of the twenty ground squirrels. The body is irregularly pyriform, but approximately bilaterally symmetrical, due to a slight indentation just behind the anterior tip of the body from which the flagella leave the body. The axostyle protrudes from the posterior tip of the body. The body length, exclusive of axostyle, ranges from 7 to 13 micra. The axostyle often extends 2 to 4 micra beyond the body. The flagella are five in number, four anterior ones which beat backwards in unison, with lashing strokes, and a *schleppgeisel*, which does not come so far forward as the others in its lashings. The flagella arise from a blepharoplast situated on the anterior surface of the nucleus. It is almost impossible to count the flagella in the living cell, because of their peculiar movements. A large number of favorable observations in the stained specimens makes it fairly certain that the number is five.

The nucleus is round, and possesses a tiny endobasal body embedded in a mass of granules (Fig. 16). Sometimes only a larger deeply staining endosome is seen (Fig. 18). About the nucleus is sometimes seen a cloud of granules (Fig. 16).

The axostyle passes from the anterior end to the posterior tip. It stains lightly. A number of favorable observations show that it originates in the basal granule.

A cytostome is often indistinctly seen at the anterior end.

Hexamitus pulcher sp. nov. This *Hexamitus*, found in eighteen of the twenty ground squirrels, is characterized by the presence in the living cell of a large number of slightly refringent spherules which stain *intra vitam* green with Janus green at first, then later are reduced to the red. These granules dissolve out for the most part in Schaudinn's fixative, although the cytoplasm of the stained cells is markedly granular (Figs. 20, 21). It was because of the wealth of mitochondria and the beauty of the vital stain with Janus green that the specific name *pulcher* was given. The body is irregularly oval, bilaterally symmetrical, and somewhat plastic. The size does not vary much from eight to ten micra in length by six or seven micra in width.

The flagella are eight in number, the anterior pair projecting almost at right angles to the body and vibrating actively in the living cell. Two other pairs are inclined to wrap themselves about the body giving it the deceptive appearance of possessing several undulating membranes. The posterior pair leaves the body at the posterior tip. Between them the posterior tips of the axostyles protrude.

Stained slides show the cell structures more plainly (Figs. 20, 21). In the anterior region on each side of the median line is an elongated, deeply staining, nucleus. Between the nuclei lie a pair of blepharoplasts from which the flagella arise, altho sometimes there seem to be two pairs. A pair of axostyles in the axis of the body terminate at about the location of the blepharoplasts. In the anterior tip of the cell is a dark spot connected to each axostyle by a fine fiber.

Summary.

The intestines of twenty ground squirrels (*Citellus tridecemlineatus*) were examined for protozoa. The following species of protozoa were found to be present: *Giardia* sp.?, *Endamœba citelli*, *Chilomastix magna* sp. nov., *Trichomonas muris* var. *citelli*, *Trichomonas* sp.?, *Tetratrichomastix* (*Eutrichomastix*) *citelli* sp. nov., and *Hexamitus pulcher* sp. nov. The first was found in the small intestine, all the others in the cœcum. In this paper all the above species except the first two are described, particular attention having been given to the morphology of the free *Chilomastix magna* and to its division phases.

So far as is known, all these protozoa are commensals. Whether or not they assist in the digestion of cellulose, which is said to take place very largely in the cœca of certain animals, is unknown.

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EXPLANATION OF PLATE.

× ca. 1950.

FIG. 1. *Chilomastix magna*, free form, showing a tuft of cortical projections in flagellar region, and dorsal view of the cytostomal structures. Large granular body dorsal to cytostomal organelles is the "parabasal body."

FIG. 2. *Chilomastix magna*, free form, dorsal view with "parabasal body" apparently following right supporting fiber of cytostome.

FIGS. 3, 4, 5. *Chilomastix magna*, free form, in division.

FIG. 6. *Chilomastix magna*, cyst.

FIGS. 7 TO 11. (Not according to scale.) Various nuclear appearances found in *Chilomastix magna*.

FIG. 12. (Not according to scale.) Dorsal view of nucleus, integrated fibrillar system and cytostomal structures of *Chilomastix magna*.

FIG. 13. *Trichomonas muris* var. *citelli*.

FIG. 14. *Trichomonas muris* var. *citelli*, dividing, nucleus in anaphase.

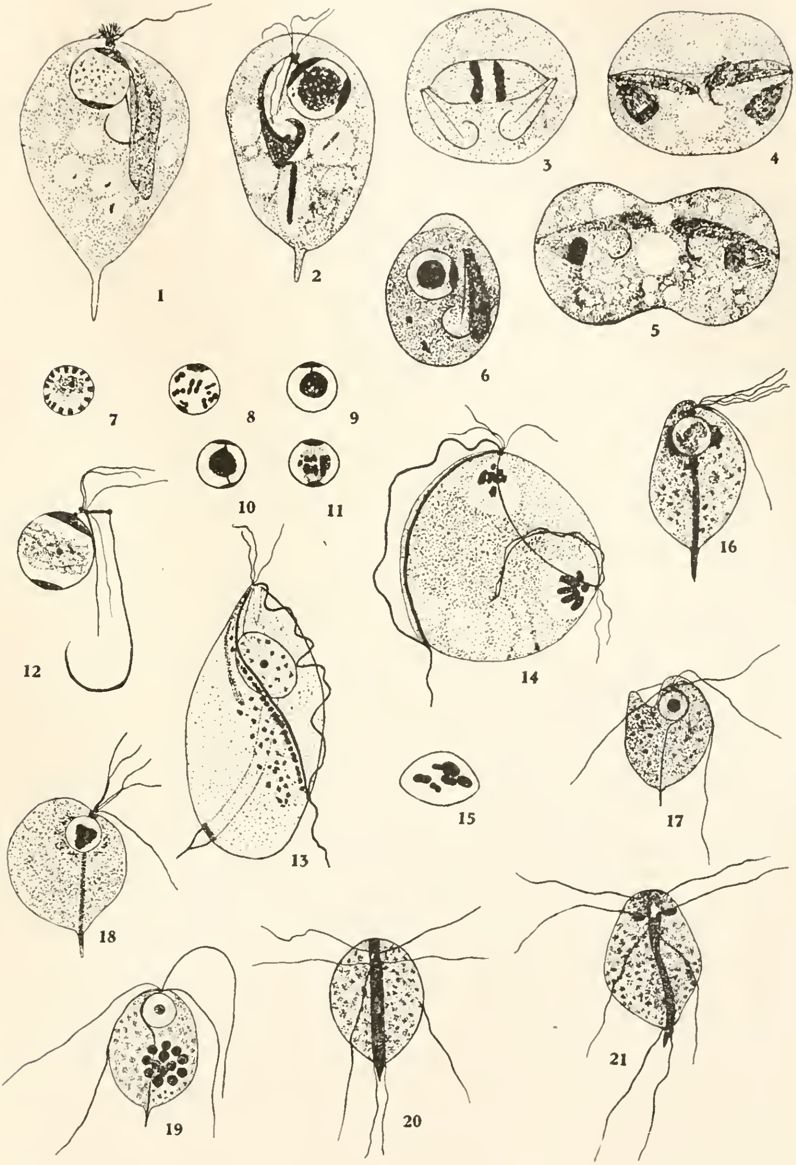
FIG. 15. *Trichomonas muris*, prophase nucleus of *Trichomonas muris* showing six chromosomes.

FIGS. 16, 18. *Tetratrichomastix citelli*, free forms.

FIGS. 17, 19. *Trichomonas* sp.? Individual in Fig. 19 infected by a *Sphærita*.

FIG. 20. *Hexamitus pulcher*, side view.

FIG. 21. *Hexamitus pulcher*, front view.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ELERY R. BECKER.

VARIATION IN THE NUMBER OF FIN-RAYS IN THREE SPECIES OF *FUNDULUS* OF THE WOODS HOLE REGION.

ROBERT PAYNE BIGELOW.

While engaged in the study of the feeding habits of young fishes in August, 1924, the author collected in Woods Hole Harbor a number of young Fundulus having a total length of from 9 to 51 mm. All except the youngest of these fish showed the darkly pigmented vertical bands, 9 to 11 in number, characteristic of the young of all three of the species of Fundulus known to occur in the vicinity of Woods Hole. (Jordan & Evermann, '96; Newman, '07; Sumner et al., '13.)

The color markings give no clue to specific identity at this stage, and the anatomical proportions, changing as they do with growth, are equally useless for identification. On the other hand, certain supporting structures, such as vertebræ and fin-rays, are developed at an early stage and remain constant in number throughout life. Therefore it was hoped that the number of fin-rays would furnish a means of assigning these young fish to their proper species.

The number of fin-rays in the dorsal and anal fins of the smallest fishes in the collection (total length 9 and 12 mm.) were found to be D 9, A 7 and 9. The two next in size (14 and 20 mm.) had in both D 11, A 10. All the others had D 13 to 15, A 9 to 11, of these seven out of ten had more than 13 dorsal rays.

Turning to Jordan and Evermann '96 for identification, the fin-ray formulæ for the species known to occur at Woods Hole were found to be given as follows: *F. heteroclitus*, D 11, A 10 or 11; *F. majalis*, D 12, A 10; *F. diaphanus*, D 13, A 11. From the fin-formulæ alone it might have been supposed that the specimens in question were *F. diaphanus*. But this was improbable because they were taken in the pure sea water of Woods Hole Harbor, whereas *F. diaphanus* is supposed to inhabit only brackish or fresh water. On the other hand, the number of rays in the dorsal fins was much greater than the numbers given for the two purely marine species.

Therefore it was decided to make a fresh examination of the fin structure in the adults of the three local species, and a thorough search of the literature on this subject. The results of this investigation are given in the present paper.

The divergence of the present results from those obtained by previous investigators may be due to the difficulties of observation. The fin-rays of Fundulus are all of the soft kind. Each ray consists of two parallel columns of segments bilaterally arranged. In the anal fin the first and last of the rays are unbranched. The other rays are branched dichotomously, one half to one third of the distance from the base, and usually the longer branches are bifurcated again. In both the dorsal and the anal fins the ray bordering the posterior margin is frequently very slender and easily overlooked. In some specimens there are two very slender posterior rays closely united at the base.

In preserved specimens that have been hardened with the fins flexed, the folding of the fin makes it very difficult to observe the posterior rays. In the present investigation observations were made with a binocular microscope and transmitted light. Some of the specimens were examined while fresh. Most of them were killed and preserved in 5 per cent. neutral formalin in sea water, after being anæsthetized by a few crystals of chloral hydrate inserted in the mouth. The specimens to be killed were placed in the formalin one at a time and the dorsal and anal fins were quickly grasped with forceps and held in an extended position until sufficiently hardened. In order that the specimens should lie flat in the dish during the remainder of the hardening process, the air-bladder was punctured by an incision in the body-wall just above the tip of the pectoral fin and parallel to its dorsal edge. By this method specimens were preserved with the dorsal and anal fins in the best posture for observation.

In the transparent fins of *F. diaphanus* prepared in this way and viewed with the binocular microscope, it was easy to count the fin-rays when illuminated by transmitted light. In the two other species pigmentation of the fin often obscured the rays. This is especially true of the densely pigmented dorsal fin of *F. heteroclitus*, on which it was at times necessary to use a dissecting needle.

The anal fin of the females of *F. heteroclitus* presents another difficulty. On the anterior edge of this fin, and extending from its base nearly half way to the tip, is a thick fold of skin enclosing the oviduct (as stated by Jordan and Evermann '96). The thickened skin extends backward over the bases of the first and second rays, at least, and may make a count impossible without dissection.

The adult material for this study consisted of 40 males and 37 females of *F. heteroclitus* and 10 males and 22 females of *F. majalis*, both species from a small salt water pond connected with the head of Woods Hole Great Harbor, and also of 10 males and 16 females of *F. diaphanus* from Oyster Pond (fresh water) in Falmouth, Massachusetts.

Although the number of specimens is too small for statistical accuracy, the results obtained show that the populations represented have considerably more variation in the number of fin-rays than is indicated by the previously published descriptions of these three species.

Full descriptions have been published by Bean, '92 and '03, Garman '95, Jordan and Evermann '96, Smith '07. In the following table the results of the present investigation are given in comparison with those of the authors just mentioned.

Present Results.						Previous Authors.			
Species.	Number.	Fin.	Number of Fin-rays.			Bean '92 & '03.	Garman.	Jordan & Evermann.	Smith.
			Range.	Most Frequent.	Mean.				
F. heteroclitus	77	D.	11–14	12	12.1	11	11–13	11	11
		A.	10–11	11	10.7	11	10–12	10–11	10–11
F. majalis	32	D.	14–16	14 or 15	14.6	13–14	14–13	12	12–14
		A.	11–12	11	11.1	11	11–10	10	10–11
F. diaphanus	26	D.	13–16	14	14.4	14	14 (13–15)	13	13
		A.	11–13	12	12.1	12	12–11	11	11

Each one of the three species has on the average a characteristic fin-pattern. In *F. heteroclitus* 12 is the most frequent number of dorsal rays, but 11 and 13 are not infrequent. One specimen only was found with 14 rays in the dorsal fin. In the anal fin 10 or 11 rays were found, the latter number having more than twice the frequency of the former.

Turning to *F. majalis*, we find a much greater number of dorsal rays, 14 and 15 being almost equally frequent. Three specimens had 16 dorsal rays. No specimen was found with as few as 13. In the anal fin the number 11 is very constant. In 32 specimens, none was found with less than 11, and 3 only with one more ray.

The third species, *F. diaphanus*, resembles the second in the structure of the dorsal fin, having usually 14 rays with considerable variation on both sides of the mean. But in its anal fin this species differs from both the others in having more rays. It almost invariably has 12 anal rays, rarely one more or one less.

Comparing the numbers reported in the literature with my findings, it will be seen that Garman's statements agree most closely with my results. The figures given in the standard manuals for the identification of fishes, like Jordan and Evermann's great work, are generally lower than mine.

The differences may be due to the difficulties of observation under less favorable conditions, or they may be due to peculiarities of the local races inhabiting the waters about Woods Hole.

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LIFE HISTORY OF *PRORODON GRISEUS.*

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Introduction.

Prorodon griseus, a Holotrichous ciliate, is somewhat plastic and variable in shape. When distended it is about two and one half times as long as wide, with its anterior end slightly wider (Figs. 1, 3). The oral aperture is sub-terminal. The anal pore is at the extreme posterior end. The pharynx is supported by twenty to thirty rod like structures. The contractile vacuole is postero-terminal. The delicate cuticular membrane is finely and closely striate longitudinally. No trichocysts are present. The cilia over the entire body are uniform in length, except at the anterior end where they are slightly longer. These longer cilia are quite active during the process of feeding. A layer of deeply stained bodies are found in the outer border of the ectoplasm. The locomotion of the expanded forms is rather smooth, rapid and straight forward, turning on their long axis. When contracted the motion is chiefly rotary.

The purpose of the following paper is to give a brief account of the more important features in the life history of *P. griseus*.

Natural History.

The material was collected from small rain pools, more especially from small hog wallows, which is one of their most favorable habitats for rapid growth and multiplication. When present in these small rain pools they are usually found in the surface film in rather large clumps, instead of being distributed throughout the water. When these small rain pools completely dry up abundance of encysted forms are found in the dry sediment on the sides and bottom. Collections were made at regular intervals (including encysted forms from the dry sediment), during the summer months of 1925. The material was placed in finger bowls with tap water added from time to time to counteract the evaporation. Few free swimming forms were found when collected. But within twenty four hours great numbers would escape from the cysts. Conjugation occurred immediately at the beginning of the free swimming stage. The period of conjugation lasts from three to four or more hours. The ex-conjugants remained in the active swimming stage indefinitely, dependent upon external conditions.

Encystment is quite common, it may occur at any period in their life history and serves more for protection in carrying them over adverse conditions, due to extreme temperature or drought. The cysts in many cases are large, spacious and permit freedom of motion within (Fig. *2a*). In the formation of the cysts the animal always contracts, assumes a spherical shape and rotates rapidly while secreting the cyst. The secretion is evenly distributed by the aid of the beating cilia. The animal usually forms its cyst in contact with some fine sediment, which acts more as a background. The cysts show considerable variation as to their flexibility and thickness (Fig. *2a–f*). The cysts are either thin or temporary and thick or permanent. The animal may remain within the temporary cyst but a short time, then escape, and immediately begin the formation of a new cyst. Large numbers of cysts are often found in immediate contact and form a sort of network, as in Fig. 2. The animal just before escaping from the cyst becomes unusually active presses against one side of the cyst wall and breaks through. The permanent cysts differ from the temporary cysts, in that they are thicker, due to the secretion of new concentric layers within the first, until there is little space left for the animal within (Fig. *2f*). After the permanent cyst is completed the animal becomes quiescent and remains in this condition indefinitely, at least until internal reorganization is complete.

In studying the formation of the cysts under the cover slip, it was found that the temporary cysts were often formed within thirty minutes, while the thick or permanent cysts required from three to five hours for their complete formation. In some of the permanent cyst under observation the animal remained quiescent for three days or more and then began a very rapid rotary motion within and soon escaped. The animal evidently must secrete some substance which helps to dissolve the cyst wall, as it becomes thinner before their escape. No experiments were made to determine how long the encysted animals were able to survive under adverse conditions. A very small part of their life history, however, is passed in the free-living condition, even under favorable conditions as verified in the laboratory.

Material and Methods.

The material was fixed at various stages in the Life history of the animal. Bouin's fluid was found to give satisfactory results. The animals were removed from the cultures with the aid of a pipette avoiding excess water, and spurted into the tubes with the killing fluid. The centrifuge was quite helpful in concentrating the animals at one end of the tube. The specimens were stained in Delafield's hematoxylin or Tulodin blue. Either stain gave good results. Erythrosin was added to the ninety-five per cent. alcohol for cytoplasmic differentiation. The animals were then dehydrated, cleared in xylol and mounted in balsam. For sections the animals were imbedded in paraffine, sectioned and stained on the slide. The sections were quite helpful in checking the results as found in the whole mounts.

Binary Fission.

Reproduction by binary fission is quite common. It may occur during encystment (Fig. 2*d*), but it is found more abundant during the free active stages. Transverse division in the encysted stage is rather difficult to verify in the whole mounts, since the division of the cytoplasm as a rule can not be recognized except in sections. Division in the free-swimming stage is more distinct and the different stages can be followed step by step. The animal shortens somewhat on its long axis. The nuclear activities (Figs. 4–6) are similar to those of the encysted forms. The micro-nucleus as is common in ciliates divides first before division of the macro-nucleus. The macro-nucleus varies considerable in shape from a spherical to a somewhat elongated or curved condition. The elongated nucleus in binary fission often becomes constricted by a nuclear cleft without any further elongation and half going to either new daughter cell (Fig. 6).

Or again the macro-nucleus elongates more and more until the halves are connected by a mere thread and finally constricts when the separation of the cytoplasm in the formation of the daughter cells is complete (Fig. 5).

The new pharynx with its rod-like structures and the new contractile vacuole are formed before the nuclei divide. The entire process of binary fission requires about one and a half hours for completion. Binary fission occurs at any period in the life cycle of the animal. But the greater number of divisions were found to occur after conjugation during the active feeding stage. But if the conjugants encyst immediately after separation, due to adverse conditions, binary fission occurs within the cyst (Fig. *2d*).

Nutrition.

The direct observation of the feeding process in *Prorodon* is rather difficult, due to the rotary motion of the individuals when stationary or when swimming. The animal feeds chiefly upon bacteria and other small unicellular organisms. The origin, formation and fate of the food vacuoles is comparable to that of other ciliates where it is more easily followed. The animal at times is so gorged with food vacuoles, that a cytological study is impossible, since the body appears as a mass of indistinctly stained objects. Many of the individuals during feeding increase from two to three times in volume before binary fission occurs. This increase in size however, is due more to expansion than growth. The large mass of food vacuoles within the organism undergoes a constant movement in one direction for short intervals and then moves to and fro as a sort of churning. This constant circulation of the food vacuoles no doubt is greatly facilitated by the constant rotary motion of the organism. The food within the vacuoles undergoes a rapid digestion or liquefaction giving the vacuoles a clear content, which soon passes out into the cytoplasm. The food vacuoles as a rule are quite large, due to the great amount of fluid surrounding the food. Thus giving the appearance of a vesicle with a clear border and a dark center. The vacuoles finally collapse and the indigestible particles are voided at the posterior end through the anal pore.

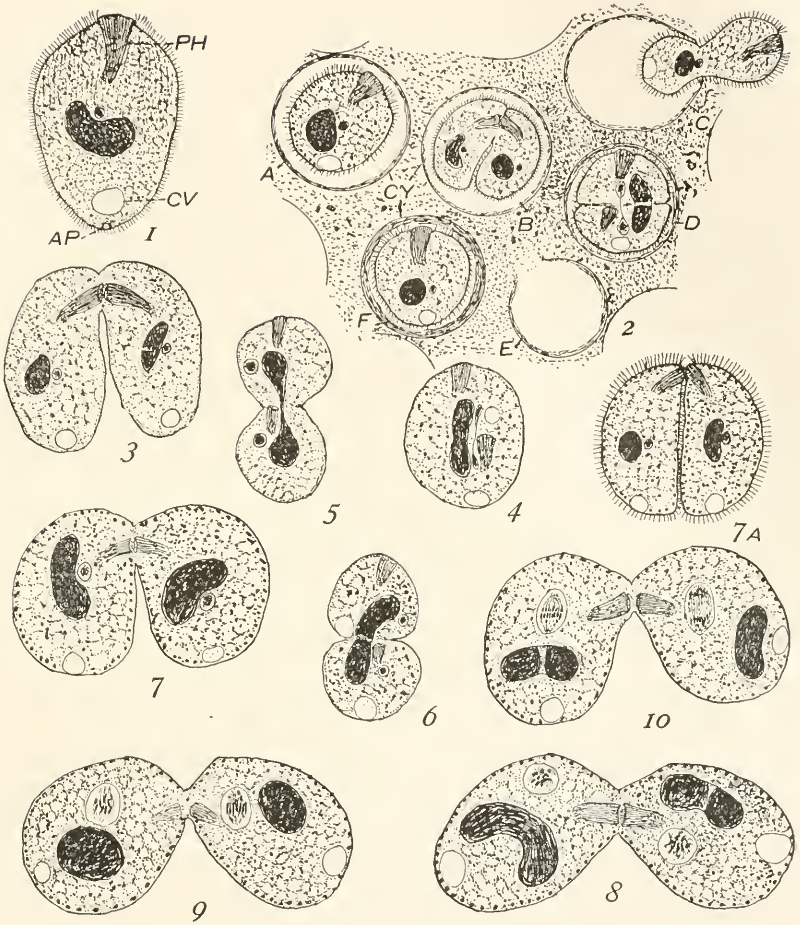


FIG. 1. Whole mount drawing of Prorodon griseus to show structure. *ph.*, pharynx; *c. v.*, contractile vacuole; *a. p.*, anal pore.

FIG. 2. Encysted forms; *a.*, temporary cyst; *b.*, conjugants within cyst; *c.*, escape from cyst; *d.*, binary fission within cyst; *e.*, cyst shell; *f.*, individual within permanent cyst.

FIG. 3. Expanded conjugants.

FIGS. 4, 5 AND 6. Different stages in the binary fission of free forms.

FIG. 7. Conjugating individuals, early stage.

FIG. 7*a*. Early conjugation before union of conjugants is effected.

FIGS. 8, 9 AND 10. Micronuclei in different stages of the first maturation division.

CONJUGATION.

Conjugation occurs shortly after the animals escape from the cysts. In the early stages of conjugation the conjugants become spherical and press closely against each other, giving the semblance of binary fission. The animals rotate very rapidly on their long axes, slightly moving back and forth on the sides in contact (Fig. 7*a*). The anterior ends of the conjugants are slightly turned towards each other, so that the free end of either pharynx comes in contact. A small cytoplasmic bridge is then formed between the two individuals in close proximity and in front of either sub-terminal oral aperture. Thus the conjugation is terminal or end conjugation instead of lateral which is more common in the ciliates (Figs. 3, 8). The animals feed but little during conjugation. The conjugants often vary considerable in size. The period of conjugation lasts about four hours. When the union of the two animals is complete they elongate, with their anterior ends turned towards each other, and swim rapidly through the water, rotating on their long axes (Fig. 3). This method of activity may continue until conjugation is complete. But the greater number of conjugants observed during the process, became quiescent usually in contact with some sediment. They assumed a spherical form and rotated quite rapidly. While in this position a temporary cyst is often formed and the conjugation is completed within the cyst (Fig. 2*b*). If the cyst however, with the conjugants is disturbed they may escape before conjugation is complete and again move freely through the culture. A second temporary cyst is seldomly formed. This process of conjugation within a cyst is often taken for binary fission and that the two daughter cells produced within the cyst conjugated before escaping. This however, is not the case, since the first steps in conjugation of all individuals, as observed under the microscope, took place in the free swimming stage. The temporary encystment of the conjugants when it occurs is secondary and not essential except in sudden adverse conditions, since it does not occur in normal conditions if the forms are continually agitated during conjugation.

The relation of the conjugants in a straight line with their anterior ends united as the figures indicate, is due more to their contraction during fixation (Figs. 8, 16). The nuclear activities during conjugation can best be followed in the encysted forms, or better perhaps in those that have just escaped from the cysts and become expanded, since they are freer from stained food vacuoles. The single micro-nucleus in the resting stage is always situated near the macro-nucleus, but never within it (Fig. 7).

First Maturation Division.

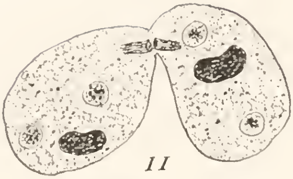
The micro-nucleus at first stains as a homogeneous mass with a dark center (Figs. 1, 7). It soon enlarges, migrates towards the anterior end and becomes vesicular with a central chromatin mass of granules on a spireme, surrounded by a large clear area (Figs. 8, 16). The chromatin becomes concentrated into eight distinct staining bodies, which become elongated (Figs. 8, 9). In the first maturation spindle fibers extend towards either pole and the eight chromosomes divide transversely, giving rise to two equal masses or daughter micro-nuclei (Figs. 10–12). Immediately after division the two micro-nuclei pass into the resting stage (Fig. 11).

Second Maturation Division.

The second maturation division is similar to that of the first. Both micro-nuclei in either conjugant undergo division. The micro-nuclei with their darker staining center become vesicular (Figs. 11, 12). The chromatin at first in an irregular spireme becomes condensed into four double-staining bodies (Fig. 13), which elongate in the form of four double rods. The reduction of eight single rods to four indistinct double rods can be considered as the reduction stage. The four bivalent chromosomes become arranged on the equatorial plate parallel to the long axis of the spindle, and apparently divide transversely instead of longitudinally, as indicated in Figs. 14 and 15. At the close of the second division as in the first the chromatin passes into the resting or more vesicular condition before the next division (Fig. 16).

Third Division.

The four micro-nuclei resulting from the second division are about the same in size when first formed (Fig. 16). The four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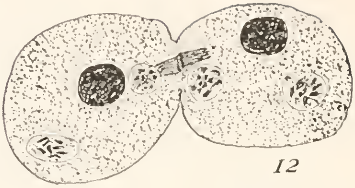
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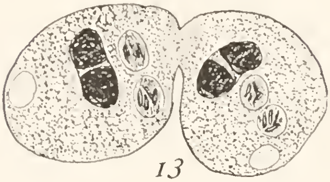
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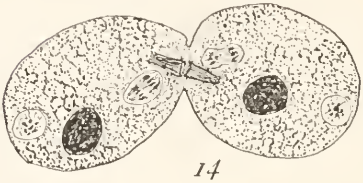
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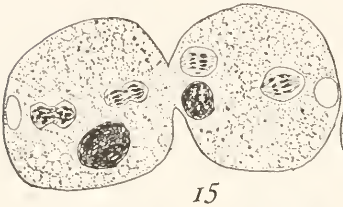
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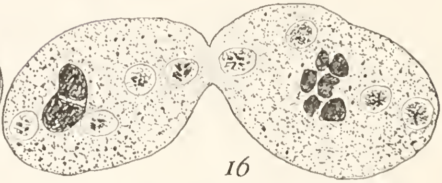
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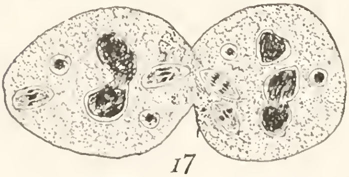
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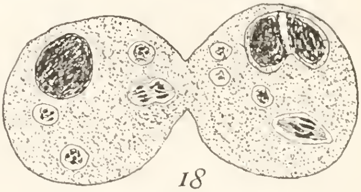
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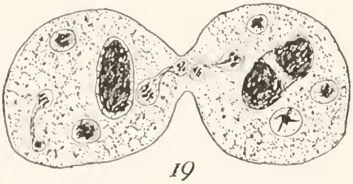
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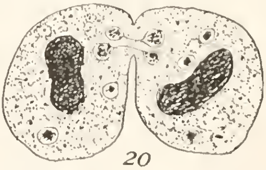
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FIGS. 11–15. Different stages in the second maturation division, showing the method of reduction of the chromosomes. Both micronuclei are dividing.

FIG. 16. Micronuclei produced by the second maturation division. The micronuclei are in the prophase stage of the third division. More than two micronuclei seldom divide in the same conjugant. The macronuclei are in the first stages of disintegration.

FIG. 17. Two of the micronuclei in either conjugant are dividing. The others are deteriorating. The macronuclei are dividing by nuclear clefts.

FIG. 18. A single micronucleus in either conjugant is dividing.

FIGS. 19 AND 20. Formation and exchange of pronuclei. The pronuclei vary in size, corresponding to moving and stationary nuclei.

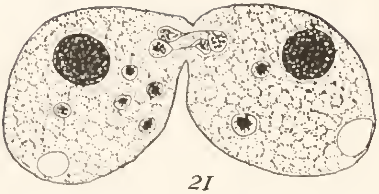
micro-nuclei of either conjugant rarely undergo division. In most cases but two of the micro-nuclei divide, and occasionally but one divides. Those that do not divide show a very dark center at first, becoming fainter, taking up less and less stain and finally disappear within the cytoplasm by absorption (Figs. 17, 19). The activities in the origin, formation and division of the four chromosomes are quite distinct (Figs. 16–18). Fig. 17 shows two micro-nuclei in either conjugant in the process of division. The others are disintegrating. Fig. 18 shows but a single division in either conjugant. While in Fig. 19 there is one in one conjugant and two in the other which are dividing. The significance of this variation in the number of micro-nuclei in the third division is still an open question for explanation. The inactive micro-nuclei may persist indefinitely before their final absorption takes place. The micro-nuclei of the third division migrate toward the anterior end of either conjugant, where they complete their division and form the pronuclei (Figs. 19–21). The third division differs from the first and second maturation divisions in that there is an elongated connecting fiber between the two daughter nuclei. This connecting fiber is not entirely a part of the spindle, but is due more to the drawing out of the nuclear membrane before separation is complete (Figs. 19–21).

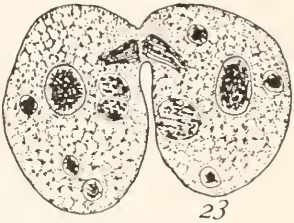
Pronuclei and their Fusion.

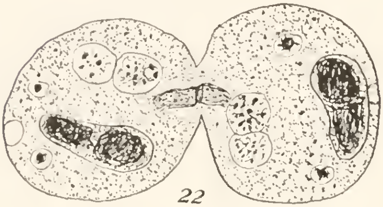
There is a slight difference of size in the pronuclei of either conjugant. The smaller can be considered as the moving pronucleus and the larger as the stationary pronucleus (Figs. 20–21). The interchange of the pronuclei is shown in Figs. 20 and 21. The fusion of the pronuclei occurs near the anterior ends of the conjugants (Figs. 22–23). In the formation of the conjugation nucleus (amphinucleus), the pronuclei at first are spherical and vesicular (Fig. 22). But later become slightly elongated. The sides of the nuclear membranes in contact may persist for awhile and prevent the direct fusion of the chromatin, giving the appearance of a double fusion nucleus (Fig. 23).

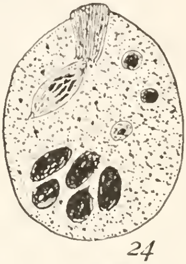
Cleavage and Formation of New Nuclei.

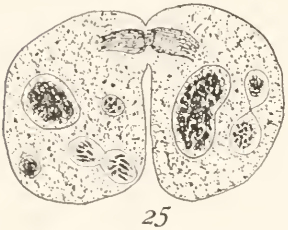
The number of divisions of the conjugation nucleus varies in different forms. It may divide once, twice, three or even four

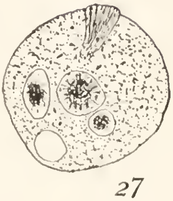


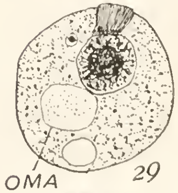


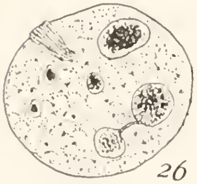


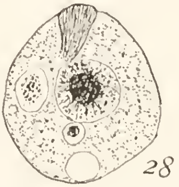












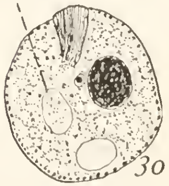


FIG. 21. Same as preceding figure with pronuclei slightly enlarged. Both macronuclei are spherical.

FIG. 22. Early stages in the fusion of the pronuclei.

FIG. 23. Later stages in the fusion of the pronuclei. The macronuclei are in their first stages of disintegration.

FIG. 24. Early cleavage stage of the fertilization nucleus. The macronucleus has divided into several parts.

FIG. 25. Later stages in the cleavage of the fertilization nucleus and the beginning of size differences in the daughter cleavage nuclei.

FIG. 26. The two cleavage nuclei remain connected by a delicate fiber in their early differentiation in the formation of the new micronucleus and the new macronucleus.

FIGS. 27–30. Different stages in the differentiation of the new micronucleus and the new macronucleus. Also the last stages in the disintegration and absorption of the old macronucleus, *o. ma.*, old macronucleus.

times, the latter case in *Bursaria truncatella*, before the differentiation of the new micro-nucleus and the new macro-nucleus occurs. In *Prorodon griseus* there is a single division. The conjugation nucleus immediately after its formation, enlarges somewhat and divides equally. The eight chromosomes are distinct and can be counted without difficulty. The two cleavage nuclei when first formed are quite similar in size and structure (Figs. 24, 25). The two daughter cleavage nuclei often remain connected by a delicate drawn out fiber, as shown in Fig. 26. One of the daughter cleavage nuclei decreases somewhat in size and produces the micro-nucleus. The other enlarges and becomes differentiated into the new macro-nucleus (Figs. 25, 26). The different stages in the formation of the new macro-nucleus are represented in Figs. 26 to 30. The chromatin network of the macro-nucleus at first is quite uniform or about the same texture throughout. The chromatin later, becomes quite distinct (concentrated) in the center, with a less dense spireme around its border (Figs. 27, 28). The dark center of chromatin enlarges until the nucleus is more or less homogeneous throughout (Figs. 29, 30). The new macro-nucleus at first is spherical, but later may assume different shapes as indicated above. The conjugants become separated during the formation of the new nuclei. According to Enriques ('08), in *Chilodon uncinatus* there is a single division of the conjugation nucleus and that the two daughter cleavage nuclei become differentiated into the new macro and the new micro-nucleus respectively.

The Old Macronucleus.

The macronucleus of the free swimming forms, as indicated above, is rather variable, ranging in shape from a circular form to an elongated condition, or at times it is somewhat curved. During early conjugation the nucleus when elongated shortens slightly and frequently divides into two or more parts (Figs. 16 and 17). But in most cases it does not divide at all (Figs. 20 and 21). The chromatin becomes more and more condensed and stains as a dark homogeneous body. The border of the dark staining nucleus fades out, leaving a dark center within a clear boundary (Figs. 23 and 25). Finally the dark center fragments into many small bodies and disappears within the cytoplasm leaving a clear unstained outline (Figs. 28–30), which is later absorbed. The old macronucleus sometimes persists in the exconjugants after the formation of the new micronucleus and macronucleus are complete, before the beginning of its disintegration.

Occasionally when the macronucleus of one of the conjugants is situated at the anterior end in close proximity to the cytoplasmic bridge as in figure fifteen, it would be carried over to the opposite conjugant, due to the movement of the cytoplasm within. The two macronuclei within the same conjugant however, pass through the usual stages in their disintegration. This passing over of the macronucleus through the cytoplasmic bridge from one conjugant to the other is quite common in one of the species of *Chilodon*, where its passage can be followed in the living forms. In *Chilodon*, however, not only the macronucleus may pass in toto from one conjugant to the other, but the macronucleus of either conjugant divides, one of the resultant daughter nuclei acting as the moving nucleus and the other as the stationary nucleus, comparable to the micronuclear activities in normal conjugation.

General Remarks.

Conjugation and binary fission of the ciliate *P. griseus*, to my knowledge, has never been described. The general activities however, in reproduction for the most part agree with the results of investigators as found in other ciliates.

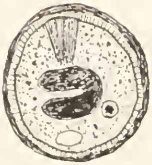
One of the chief points of interest in studying the different stages in the life history of the ciliate *P. griseus*, is the direct association of binary fission and conjugation with encystment. The entire process of cell division may occur within a given cyst. While the first steps in conjugation always occur (as far as my observations go), in the free swimming forms and encysts later when the union of the two conjugants is complete. Temporary encystment however, can not be considered as essential for binary fission and conjugation. Since both may occur in the free forms. Encystment here acts more as a protection in unusual or adverse conditions. In no instance observed did the daughter cells produced by binary fission within the cyst, undergo conjugation before their escape.

P. griseus differs from most Holotrichs, in the first stages of conjugation, in that each conjugant contracts, becomes sperical, and rotates vigorously. The sides in contact are somewhat flattened, with their sub-terminal oral apertures facing each other. They remain in this spherical shape until a point of cytoplasmic union is complete (Fig. 7*a*). Then they elongate (Fig. 3), and move freely through the culture until conjugation is complete. A greater number of the paired conjugants as a rule remain contracted, rotate on their long axes and secrete a temporary cyst in which they remain for the greater part or the entire period in the completion of conjugation (Fig. 2*b*).

In this connection it may be of interest to note that no conjugating individuals were found out of doors or under wild conditions. Although many of the pools were found in unfavorable conditions due to evaporation. In fact very few free-swimming forms were found at any one time when collections were made. Most of the individuals were encysted. But when placed under laboratory conditions, within a few hours the greater number of individuals escaped from the cysts, and became quite active. This was followed immediately by a regular epidemic of conjugation. Very few single individuals could be found in the cultures. Again within three to five hours very few conjugating individuals were present. The exconjugants during their free-swimming period fed quite actively and reproduced by binary fission. In about five hours encystment again occurred. Thus beginning a new cycle. A few active forms however, can be found in the cultures at all times.

It is maintained by Calkins and others that encystment (especially where cellular reorganization occurs), and conjugation are both conducive to renewed vitality. While by Mast and others it is held that neither encystment nor conjugation have any appreciable effect in producing new vigor.

In *P. griseus* however, we have conjugation immediately following encystment or even the conjugants may encyst during their nuclear exchange and reorganization. Here we might conclude that the exconjugants would receive unusual renewed vigor and increase the fission rate. This however, is not what we find in *P. griseus*. It is true that an epidemic of cell division takes place in the exconjugants. But if we isolate an equal number of the exconjugants that encysted during conjugation, and an equal number of those that failed to conjugate immediately after encystment. We find that the rate of fission per given number is about the same in the two isolated lots. Hence it may be safe to conclude that simultaneous encystment and conjugation do not produce renewed vigor or at least increase the fission rate in *P. griseus*.



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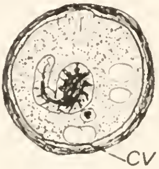

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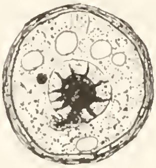

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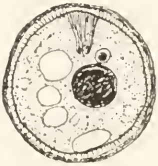

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FIG. 31. Prorodon griseus in the early completion of the permanent cyst with all the organs in tact.

FIG. 32. Later stage of encystment with the disappearance of the cilia and the breaking up of the pharynx. The micronucleus and the contractile vacuole(?) persist. The macronucleus is in its early stage of metamorphosis, *c. v.*, contractile vacuole.

FIG. 33. The macronucleus in its later stage of metamorphosis. Several cytoplasmic vacuoles are present.

FIG. 34. Final stages of metamorphosis of macronucleus or cytoplasmic reorganization of structures during encystment. The missing organs have reformed. The new macronucleus is a direct metamorphosis of the old.

Encystment in the ciliates according to Calkins may serve a threefold purpose. That is for protection, reproduction and reorganization. In *P. griseus* there are two types of cysts which we have designated as thin or temporary and thick or permanent. The two types differ in the thickness and the compactness of their walls. The temporary cysts are quite flexible and yield to the moving ciliate within. The temporary cyst serves two purposes: first for protection against sudden adverse conditions such as temperature, scarcity of food and evaporation. The ciliate if disturbed may remain in the temporary cyst less than an hour, and immediately after escape form a new cyst. The organism during temporary encystment does not undergo any internal changes. It maintains its spherical shape and continues to rotate. The second purpose is for reproduction by transverse division. Likewise conjugation, except the early fusion of the conjugants, may occur within the cyst. The third purpose is for the reorganization of the cell. The cyst in this case is of the permanent type, its walls being thick and dense. The individual becomes quiescent, and loses its cilia and pharynx during internal reorganization (Figs. 31–33). In this latter case the organism can not be recovered from the cyst within a period of at least four or more days or until its reorganization is complete.

The micro-nucleus and contractile vacuole persist throughout the reorganization without undergoing any appreciable change. The macro-nucleus however passes through a distinct series of changes as indicated in Figs. 31 to 34. Fig. 31 represents the condition at the beginning of encystment when all of the organs are present. The macro-nucleus is horse-shoe shaped, but in many individuals it is spherical. In the first steps of macro-nuclear metamorphosis the chromatin becomes ragged (Fig. 32), with a clear border beneath the nuclear membrane. The central region of the chromatin remains dark. Fig. 33 shows a third stage in which the chromatin has taken a more spherical shape with the ragged edges fraying out. Finally in Fig. 34 the macro-nuclear change is complete, the more central portion of the chromatin persisting as the new nucleus. The organs which were lost have reformed.

The macro-nuclear changes in *P. griseus* are similar to the conditions found by Stolte, in *Blepharisma undulans* and by Brand, in *Vorticella microstoma* and in *Stylonychia mytilus*. The macro-nuclear activities during encystment are considered by them as a cytoplasmic reorganization process since no new nuclei are formed. According to Stolte cytoplasmic depression, occasioned by adverse external conditions leads to encystment, and nuclear depression leads to conjugation. In *Didinium nastutum* however, as reported by Calkins and in *Stylonychia pustulata* as reported by Fermor, the macro-nucleus disintegrates and a new micro-nucleus and a new macro-nucleus are formed from the old micro-nucleus, thus undergoing a complete nuclear reorganization comparable to conjugation.

General Summary.

1. *Prorodon griseus* is a Holotrichous ciliate. It is recognized by its sub-terminal oral aperature, its rod like structure enclosing the pharynx, and its terminal contractile vacuole.

2. *P. griseus* has a single micronucleus and a single macronucleus.

3. Binary fission and conjugation occur either while encysted or in the free forms.

4. Encystment serves for protection, reproduction and reorganization.

5. Cysts are of two kinds: (*a*) temporary for protection during short intervals. (*b*) permanent for reorganization of cell and protection for long intervals, from one cycle to the next.

6. Conjugation is terminal, fusion occurring at their anterior ends. Conjugation occurs immediately after the animals escape from the cysts.

7. The animals are found encysted during the greater part of their life history. The greater number of free forms are found immediately after conjugation, during the active feeding period.

8. Encystment and conjugation in *P. griseus* have little or no effect in the production of new vigor and in the change of fission rate.

9. The old macronucleus persists during early conjugation, then disintegrates and disappears by absorption within the cytoplasm.

10. The new micronucleus and macronucleus are formed from the daughter nuclei, produced by a single division of the conjugation nucleus.

11. The reduction of chromosomes occurs in the second maturation division by a pairing of the eight chromosome in the formation of the four bivalent structures.

12. In the third division the pronuclei at first are connected by a drawn out fiber.

13. The pronuclei elongate slightly during their process of fusion.

14. In cellular reorganization within the permanent cyst, the cilia and pharynx disappear. The micronucleus and contractile vacuole (?) persist. The macronucleus passes through a distinct metamorphosis.

15. Reorganization during permanent encystment in *P. griseus* is of the cytoplasmic or passive type. Nuclear reorganization or formation of a new micronucleus and a new macronucleus from the old micronucleus was not found to occur.

Columbia, Mo.,
July 6, 1925.

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STUDIES IN THE LIFE HISTORY OF *EUGLENA*.*

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INTRODUCTION.

Attention has been called many times to the importance of research on the Flagellates because of their relatively simple structure, their apparent position at the bottom of the scale of living organisms, their partaking in many instances of characteristics of both plants and animals, their universal distribution and their contributions to our knowledge of general principles of biological phenomena. Many workers have engaged in such investigation and much progress has been made.

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The present investigation was undertaken on the Euglenoidina because of the primitive character of the organisms composing the group, their wide distribution, their general use as types in fundamental courses in biology and the fact that the literature does not show a complete and critical account of the cycle of any one of them with attention given to all of the organoids of the cell during the successive stages in the cycle.

Solutions have been sought to four main problems through the study of:

(1) the origin and behavior of the chromatic elements of the cell;
(2) the nature and behavior of the endosome of the nucleus;
(3) the origin and behavior of the locomotor apparatus during mitosis;
(4) the location of the division center.

Acknowledgments.

The desirability of critical work on *Euglena* was originally suggested to me by Professor Robert C. Rhodes of Emory University in 1919. I desire to express my appreciation to him for much of the earlier criticism and suggestions as well as for the original plan of the work. The investigation was completed under the direction of Professor Gary N. Calkins of Columbia University. I am indebted to him for his suggestive interpretations and criticisms, both constructive and destructive, as well as for his continued encouragement and inspiration, beginning at Woods Hole in 1922, continuing at Columbia during 1924–25 and at Woods Hole in the summer of 1925. I am especially indebted to my wife, Bernice Hall Baker for her painstaking care in the preparation of the figures.

Material and Methods.

Several species of *Euglena* from wild cultures have been collected and identified. Attempts have been made to cultivate all of them in order to secure material for detailed study. It has been found however, that a wide variation exists in the type of culture medium required for the different species, and that when one says a culture medium has been discovered for *Euglena* the statement means little unless the species is indicated.

A small amount of green scum known to contain *Euglena*, found on the surface of a culture of pond water, leaves and grass, was placed in a large petri dish containing one liter of tap water and one gram of malted milk. An abundance of active *Euglenæ* were produced in a few days and identified as *Euglena agilis*. From this original culture all the material for my sub-cultures used in the investigation were secured.

The most satisfactory culture medium for this species has been found to be split pea infusion made by boiling forty split peas in one liter of tap water. Sufficient citric acid is added to keep down extensive growth of bacteria. For keeping cultures in an inactive condition, quince seed jelly as recommended by Turner (1917), has been used. This medium has also been satisfactory for the study of the animals in the living state under the microscope. Inoculation of split pea infusion with material from the quince seed jelly produces abundant active forms within a few days.

Various killing fluids have been used, the best results being obtained with Schaudinn's fluid heated to 56 degrees Centigrade. Other fluids used were Flemming's strong, Bouin's picro-formol solution, chrom-acetic-osmic mixture, Zenker's fluid and osmic acid vapor.

For staining, aqueous iron-alum hæmatoxylin, long method, has given the most satisfactory preparations. The use of a counter stain has aided in the study of the motor apparatus. Eosin and bordeaux red (.1 per cent. solution for 24 hours before mordanting) have given the best results. All figures have been drawn from material counter-stained with eosin.

Other stains used were Delafield's hæmatoxylin, safranin and light green, Flemming's triple, Borrell's stain, and iron alum hæmatoxylin counter stained with light green or orange G.

The organisms were killed at different times during the day, the most satisfactory material being secured from eight to twelve

P.M. During that period the divisions stages are the most numerous. Twenty cc. of the material was taken at thirty minute intervals, centrifuged, killed, and either stained en masse or taken from the centrifuge tube, placed on cover glasses and stained according to that method. The first method gives good results when the material is abundant. Material killed at ten P.M. gave the greatest number of stages of mitosis. After twelve P.M. most of the divisions initiated around eight P.M. have been completed, and from twelve P.M. to six A.M. very few dividing forms can be found in the culture.

The animals in a culture appear to be active during the day but usually about five P.M. they become less active, some assuming a spherical shape then extending the body again and again as if undergoing intense metabolic activity. This difference in the cyclical phases of *Euglena agilis* as observed at different times of the day, and especially the nocturnal incidence of dividing forms may be explained perhaps by the difference in the carbon dioxide content of the medium at different times. At evening and during the night the photosynthetic processes which have gone on during the day are retarded and cease. The normal activity of the animals would then increase the relative amount of carbon dioxide present and perhaps influence the changes which are observed. Sufficient data have not yet been obtained to prove the validity of such an assumption.

General Morphology.

The description of *Euglena agilis* as given by Carter and quoted from Kent is as follows:

"Body somewhat flask-shaped, inflated and widest posteriorly, attenuate anteriorly; a short, blunt caudal prolongation sometimes present, but not essential; multiplying in the active condition by longitudinal and transverse fission, and in its passive or encysted one by crucial or by linear segmentation; color green, movements very active. Length 1/600 inch."

The species used in the present study conforms to this description except for the fact that it does not divide by transverse fission. In addition there are certain characters observed which seem to be diagnostic:

(1) three to seven oblong, flattened chromatophores present, each with an uncovered pyrenoid in the center;
(2) the periplast is more rigid than in many other forms studied so that the body does not show typical euglenoid movement when in the active condition;
(3) flagellum about two thirds the body length;
(4) paramylon bodies small and not very abundant.

Gullet and Reservoir.

This structure has been described and figured for many flagellates, including *Astasia ocellata* and *Euglena viridis* (Khawkine, 1886); *E. viridis* (Wager, 1899); *E. sanguinea* (Haase, 1910); *E. Ehrenbergii* (Hamburger, 1911); *Astasia levis* (Belar, 1916); *Peranema* (Hartmann and Chagas, 1910); *Eutreptia* (Steuer, 1904); *Copromonas* (Dobell, 1908); *Menoidium* (Hall, 1923).

In *E. agilis* a somewhat funnel like depression is located at the anterior end and leads directly into the gullet which curves as it passes inward toward the side of the animal on which the stigma is located. The reservoir seems to be in direct connection with the gullet; it is asymmetrical in shape, the largest portion being toward the inner side of the body (Fig. 1). The vacuole system in connection with the reservoir has not been made out in E. agilis but in some of the larger species examined, *E. polymorpha*, *E. caudata*, *E. sanguinea* and *E. spirogyra*, a series of small pulsating vacuoles have been seen discharging their contents into the reservoir along its periphery.

The function of the gullet-reservoir system is not definitely known. Khawkine (1886) suggested that it was used for the admission of liquid food, giving as evidence the accumulation of small granules of paramylon near the gullet when the animals were fed potato starch in the dark. Kent describes observations made on *Euglena viridis* kept in carmine suspension, where he observed the passage of fine particles of the carmine into the body by way of the gullet. Wager (1899) considers it an excretory system. Rhodes and Kirby (Mss.) find that *Hetronema*, a related form, lives on a diet of *Euglena proxima*, which they have observed being taken in through a specially modified cytostome supported by a horseshoe-shaped lip and two deeply staining pharyngeal rods. This structure is separate and distinct from the gullet-reservoir system as described in *Euglena*. They conclude that the reservoir functions primarily in the reception of the discharge of contractile vacuoles which are located on its periphery. Our own observations indicate that all these functions may be exercised by the system in *Euglena*.

The Nucleus.

The nucleus of *Euglena agilis* lies at the center of mass of the body, being located in the region of the center of the posterior flask shaped enlargement. It is spherical to oblong or ovoid in shape. Its chief characteristic is the presence of a large, deeply staining, central body or endosome, the "Binnenkorper" of Doflein and Tschenzoff. Immediately surrounding the endosome is a light hyaline area apparently devoid of chromatin material. The presence of this hyaline layer enables one to observe more readily the budding off of the motor complex, as described below, from the endosome during the early stages of mitosis.

In the resting nucleus, chromatin granules arranged apparently at the nodes of a linin network, surround the hyaline area. During the early prophase of division distinct chromosomes are formed by the growth and aggregation of these chromatin masses. When preparations stained in iron-alum hæmatoxylin and counter stained with eosin, are destained considerably, the endosome retains the hæmatoxylin so as to appear quite dark while the chromosomes appear red with the eosin. This seems to indicate that there is a great deal of linin in the network on which the chromatin masses are lodged. In most instances the masses seem to occur in pairs giving the appearance of dumb-bells. Wenrich describes somewhat the same appearance of the chromatin in *Trichomonas*. He says that in the anaphase each chromosome becomes constricted and passes into the resting nucleus as a paired structure.

A nuclear membrane can be distinguished with difficulty with the stains used, yet the shape of the nucleus and its apparent separation from the cytoplasm certainly indicates its presence as well as its persistence during mitosis.

The endosome appears homogeneous, although vacuoles or light areas which appear as vacuoles, of varying number and distribution, can be observed at all times during the cycle. No centriole has been observed within the mass.

The Chromatophores.

The presence of chromatophores is characteristic of all but two species of *Euglena* heretofore described. They occur in varying numbers in the different species and are usually described as being of characteristic shape for each species. In *E. agilis* they appear differently at different stages in the cycle. In the resting stages they are rod shape, arranged parallel to the long axis of the body, and with a distinct pyrenoid in the center. During the stages preparatory to division as well as during the reconstruction of the daughter cells after division, they appear to be more rounded or oblong, much larger and with the pyrenoid quite indistinct or apparently absent. The number likewise is variable, since they are apparently distributed to the daughter animals in some cases before the cell divides and in other cases they divide before cell division and thus give a larger count in the body. These facts lead one to believe that this feature of the cell of *Euglena* is not a satisfactory basis for the classification of the group. A few preliminary observations on the effect of varying H-ion concentration in the culture medium indicates that the form, number and arrangement of the chromatophores is affected by changes in the culture medium. More work needs to be done on this point.

The Motor Apparatus.

This consists of a single flagellum and related structures as described below. It extends through the gullet, usually lying on the side nearest the stigma or eye-spot, and ends at one side of the lower border of the reservoir in two distinct branches, each terminated by a granule. The two branches are unequal in size and structure and perhaps also in function. The branch nearest the side of the gullet and reservoir is larger than the other and has a lens shaped enlargement at the point where the gullet widens into the reservoir, apparently underneath the stigma. This arrangement is similar to that described by Wager for *E. viridis*. The continuation of this branch appears to form the external flagellum. The smaller branch passes from its anchoring granule through the reservoir to a point opposite or above the lens-shaped enlargement on the larger and there fuses with it. In the early stages of development after division the two branches appear to be entirely separate, though the larger grows more rapidly and extends from the cytostome before fusion with the smaller. In some few cases observed there is an indication that the two branches may remain separate not only at the outset but permanently. The granule at the base of the larger branch seems to originate first from the kinetic center and is therefore interpreted as the blepharoplast. This divides and gives rise to the granule at the base of the smaller branch which granule is here designated the basal granule.

During the late prophase and continuing for some time after division is completed there may be seen a rhizoplast extending from the blepharoplast into the cytoplasm and ending on the nuclear membrane in a heavily staining body which originates in the early prophase from the endosome. In older animals such a connection cannot be detected, indicating that direct connection with the nucleus is not necessary for the proper functioning of the motor apparatus. The early connection of the various parts seems sufficient grounds however, to designate the motor apparatus of *Euglena agilis* as a neuro-motor system as defined by Kofoid and his students. Their definition involves an integrated motor system connecting with the nucleus. (Kofoid and Christiansen, 1915*a*, *b;* Kofoid, 1916; Swezy, 1916; Boeck, 1917; Kofoid and Swezy, 1919*a*, *b*, 1920, 1922; Hall, 1923.) It is true that only during the later division stages and for a short time after binary fission there is a complete integration of nucleus and motor apparatus, even to the presence of an intra-nuclear rhizoplast (Fig. 13). The presence of these rhizoplasts is merely reminiscent of the process of development of the parts of the motor apparatus, as will be described later, since they are not permanent structures of the cell.

REPRODUCTION.

The typical method of reproduction as described for Euglenidæ is by longitudinal fission, accompanied by division of the nucleus and varying behavior of other organoids of the cell. This division occurs in the motile state in some species, in a quiescent but not encysted condition in others while in some a true cyst is formed before the organism divides. In the encysted condition the division may be binary or multiple. Some of our observations indicate that some species may divide in the free swimming condition under proper conditions but when the conditions arise which induce encystment, these conditions being either internal or external or both, the divisions may take place in the cyst. The special problems of encystment are now under investigation and will constitue the subject matter for a later paper. In this paper we are concerned with the details of the division process in *Euglena agilis.*

Nuclear Division.

Three principal changes characterize the beginning of nuclear division:

(*a*) changes in the endosome and the budding off of the kinetic complex;
(*b*) formation of the chromosomes;
(*c*) migration of the nucleus anteriorly.

(*a*) *Changes in the Endosome and the Budding off of the Motor Complex.*—The endosome seems to become more vacuolated than in a typical vegetative stage, increases in size, and at the same time a small knob or bud appears on one side (Fig. 2). The bud usually appears on the side of the endosome toward the anterior portion of the animal but in several instances it has been observed first in other positions becoming turned anteriorly during the migration of the nucleus. The bud increases in size, becomes a spherical or slightly elongated, deeply staining mass and passes through the hyaline area surrounding the endosome, thence through the chromatin of the outer nucleus to the nuclear membrane. In most instances it can be seen definitely connected to the endosome by a rhizoplast (Figs. 3, 4).

On the nuclear membrane, the mass, now a distinctly spherical body, divides and the daughter bodies separate and pass gradually to opposite sides of the nucleus (Figs. 4, 5, 6). Later the portion of the nuclear membrane which lies between the bodies is drawn into a fairly definite line when viewed in optical section (Fig. 8).

As they separate the bodies again divide giving rise to the blepharoplasts of the two future cells. From each blepharoplast a new flagellum arises. When the nucleus has passed anteriorly until it comes into contact with the lower border of the reservoir, each blepharoplast divides, giving rises to a basal granule which serves as the basis for the smaller branch of the bifurcated flagellum (Fig. 6). In some cases the mass from the endosome does not follow this sequence, but both roots of the flagellum begin to grow out directly, the blepharoplast and basal granule forming later. This is much as described by Hartman and Chagas for *Spongomonas*.

(*b*) *Formation of Chromosomes.*—The chromatin which in the resting nucleus is observed at the nodes of a linin network in the form of granules and masses of various sizes, now collects in a number of separate rods or filaments, the chromosomes (Figs. 3, 5). The entire nucleus stains more heavily at this time than in the vegetative stages, and gives the appearance of containing more chromatin. It is perhaps true that the chromatin masses increase in bulk and undergo chemical transformation so as to give the changed appearance. The dumb-bell shape observed in the resting nucleus may still be observed, as the chromosomes become outlined. Each is seen more or less constricted at first, later becoming almost uniform in width. All the chromosomes do not form simultaneously, hence the areas where the separate chromatin masses are beginning to fuse to form the chromosomes show what might be interpreted as a spireme structure. Free ends may be distinguished however, so that one is led to believe that a continuous spireme is not formed but rather each chromosome retains a certain degree of continuity or individuality which has persisted throughout the resting stages, the chromosome as such reforming in the prophase from the chromatin network.

As the filaments or rods assume definite outlines they are seen to be more or less looped. This appearance is perhaps due to the fact that the chromatin has increased in bulk while the nuclear membrane has persisted and has not enlarged in proportion. The looped shape might easily be mistaken for a paired arrangement of separate units, especially as the chromosomes come to lie in the equatorial region and the ends of the loops become pressed close together (Fig. 7).

(*c*) *Migration of the Nucleus.*—While the changes described above are taking place, the body of the animal tends to become alternately rounded and extended, during which movement the nucleus passes anteriorly until it comes into contact with the lower border of the reservoir. Here the nuclear membrane and the border of the reservoir present a thickened appearance and because of the presence of the kinetic elements at opposite sides of the nucleus might easily be mistaken for a paradesmose (Fig. 8). Such an interpretation has been given to *Euglena agilis* by Hall (1923). A careful study of these stages has been made and in no case has a definite line been detected other than would be the natural consequence of the coming together of two surfaces such as the nuclear membrane and the border of the reservoir.

By the time the nucleus has reached the reservoir, the endosome has elongated, passing from spindle to rod shape, then to dumbbell shape. In the meantime the chromosomes collect in a more or less haphazard fashion along the middle portion of the endosome. In the main they assume a radial arrangement as in Fig. 7. In this condition each chromosome shows a definite longitudinal split.

(*d*) *The Metaphase.*—The chromosomes, many of which show a longitudinal split during the prophase, and most of which are in the form of loops, now show a distinct separation along the plane of the longitudinal split. The separation of the split halves produces a complicated figure. Those which appear as loops open out from the apex first, gradually assuming a diamond or double V shape. Those not as loops begin to separate at one end first and gradually form elongated single V shape structures which extend the entire length of the equatorial cylinder. As the splitting proceeds the halves come to lie more nearly parallel to the elongating endosome and produce an apparent equatorial cylinder (Fig. 10). This parallel arrangement of the chromosomes with their subsequent complete separation in the midline might easily be mistaken for a transverse division.

The endosome assumes a single bend or curve in its middle region with its convex side toward the posterior part of the body of the animal. This is perhaps caused by the increase in its length beyond the width of the body of the animal, the bend being the means of adjustment to the width of the body. On the other hand the bend may be the first indication of the plane of separation of the daughter animals and brought about by the metabolic changes in this plane (Fig. 12).

(*e*) *The Anaphase.*—During the anaphase the endosome continues to lengthen, accompanied by the nuclear membrane and motor complex on the outside. The ends of the endosome increase in size, perhaps at the expense of the mid-portion or centrodesmose. This region becomes much attenuated and is seen to lose its staining capacity for hæmatoxylin and to take the eosin in its place. The nuclear membrane can be followed distinctly between the groups of daughter chromosomes and seems to inclose a plastin like spindle. Material counter stained with light green does not show definite fibers in this spindle but indicates, rather, protoplasm that has been drawn out under the influence of the division forces.

The opposite ends of the endosome are now seen to be quite unlike in shape. One end is club shaped and rounded, as in the preceding stages, while the other is elongated anteriorly and flattened on the outer side. A projection can be detected pointing toward the granule on the periphery of the nucleus on that side (Fig. 11). In many figures a rhizoplast may be traced from the granule to this projection. This marks the path of the budding off of the original motor complex as observed in the early prophase.

The daughter chromosomes formed in the late metaphase or early anaphase now pass toward opposite ends of the nucleus and group themselves in a sort of barrel shaped cylinder around the enlarged ends of the endosome. The ends of the endosome project beyond the cylinder of chromosomes into a clear hyaline area (Fig. 11). The number of chromosomes seems to be equal at both ends and can be counted with reasonable accuracy. A number of counts from figures in end view disclose approximately 14 distinct chromatin rods.

(*f*) *The Telophase.*—The telophase begins after the endosome has elongated until the thin mid-portion of the centrodesmose becomes constricted. The nuclear membrane is likewise constricted and the daughter nuclei begin to round up. Here some interesting information can be had regarding the development of the motor or kinetic complex. Each nucleus is seen to be somewhat pear shaped, its apex turned toward the border of the new reservoir and usually in contact with it (Fig. 13). At the apex of each nucleus there appears the deeply staining granule which has given rise to the blepharoplast of the new flagellum, as described above. These granules have arisen by division of the bud which came from the endosome in the early prophase. The rhizoplast which marks the path of origin of the bud from the endosome may be seen as a persistent structure in one of the nuclei. From each granule can be seen a rhizoplast passing upward to the lower border of the reservoir and ending in the blepharoplast. From the blepharoplast a connecting rhizoplast is seen passing to the basal granule of the smaller branch of the flagellum. From both the blepharoplast and basal granule the new branches of the flagellum may be seen passing upward through the reservoir into the gullet.

The endosomes of the daughter nuclei become rounded; the chromosomes take on a somewhat spiral arrangement in the peripheral region of each nucleus; condensed nodes appear in each chromosome, giving to them a beaded appearance. Each definite chromosome loses its distinct outlines and gradually becomes a part of the rapidly forming network of chromatin characteristic of the vegetative nucleus.

During these reorganization stages of the telophase, the chromosomes seem to change their staining capacity. Each appears more diffuse and broader. This appearance may be variously interpreted. In the first place it is conceivable that the change is due to a longitudinal splitting of each chromosome, the halves remaining close together without separation before passing into the resting condition. This is the interpretation which Tschenzoff gives for *Euglena viridis*. Another explanation is that the metabolic changes in the chromosomes during the reorganization period, especially the relative change from gel to sol, is sufficient to give the diffuse appearance.

Division of the Gullet and Reservoir.

As has been mentioned above, the nucleus migrates anteriorly during the early prophase and comes into contact with the lower border of the reservoir. The mass of the kinetic complex has in the meantime divided and the daughter halves have passed away from each other on the upper margin of the nuclear membrane. The lower border of the reservoir is seen to broaden as the separation of the masses or granules continues, much as if they were the centers of the division process.

A small lobe is seen to form on each side of the widening reservoir in contact with the nucleus (Fig. 6). As the nucleus elongates the lobes increase in size and eventually become separated in the anaphase (Fig. 10). This separation continues upward along the gullet until two structures are formed from the original one, remaining connected however, at the peripheral opening of the original gullet. In the early telophase the external opening becomes oval shaped and gradually divides.

No indication has been observed of the disappearance of the old gullet-reservoir and the rebuilding of a new one as Dobell describes for *Copromonas*. Neither has one daughter animal been seen to take over the old system while the other animal builds a new one, as described by some authors.

Division of the Body.

The first indication of the longitudinal split of the body is seen in the region of the reservoir (Fig. 13). The split continues posteriorly along the median plane of the body until the two daughter animals are completely separated. The flagella appear extended from the gullets at an early stage after separation begins, and their movement perhaps aids in the eventual pulling apart of the bodies. Observations of living animals at this stage shows continued twisting and writhing of the anterior ends as separation progresses, until the two bodies are connected by a small strand of protoplasm. Then usually both animals take a position in a straight line with their anterior ends pointing in opposite directions, in which position a veritable tug of war takes place until the bodies are completely separated. Immediately after separation the bodies round up slightly, forming elongated oval shapes and remain more or less quiescent for some minutes. In this quiescent condition the final processes of reorganization seem to occur.

Observations of the pairs of daughter animals in this quiescent period immediately after separation shows interesting changes in the relationship between the nucleus and the kinetic complex. The deeply staining granule at the apex of the pear shaped nucleus becomes surrounded by a lightly staining halo, increases in size, and begins to show indications of breaking away from the nuclear membrane (Fig. 15). In the meantime the nucleus begins to rotate slightly and passes backward toward the posterior region of the body. When this posterior migration begins the rhizoplast connecting the granule with the blepharoplast, and in one of the pair of daughter animals an intranuclear rhizoplast as well, can usually be distinguished. As the animals begin to elongate after the quiescent period the granule becomes disconnected entirely from the nuclear membrane and passes with its surrounding halo into the cytoplasm. The rhizoplasts may be seen for some time after the separation but they eventually disappear and the granule becomes entirely separated from the motor organs and the nucleus (Figs. 16, 17).

The separated granule seems to have no definite position in the cytoplasm, but is found in various places. It remains as a definite structure until the later stages of the next successive division when it is observed to gradually fade out of view. In some cases it has been observed as late as the quiescent period after binary fission, so that two such bodies are then found in one cell. These cases however, are exceptional.

Behavior of Chromatophores During Mitosis.

When the division process is first initiated in the animal, the chromatophores are quite definitely delineated. A pyrenoid lies in the center of each one and takes a fairly intense stain with the hæmatoxylin. The body of the chromatophore stains with the eosin rather than the hæmatoxylin. In material counter-stained with Orange G the pyrenoids become a light orange yellow. In Borrel's stain the body of the chromatophore becomes green while the pyrenoid is blue. As the division process goes on the pyrenoids show definite changes in contour and apparently in composition. The chromatic substance becomes first aggregated around the periphery in the form of distinct masses, which later become elongated and thread-like. In the meantime the body of the chromatophore loses its definite outline, becomes more rounded and apparently increases in size. The individuality of the pyrenoid becomes less distinct, until the later stages show the chromatophore as more less homogeneous with a slightly denser central region. This central region increases in size, elongates, becomes constricted in the center and forms two regions from which the pyrenoids seem to form in the reorganization period of the cell. This division of the chromatophores is not synchronous with the division of the nucleus and the cell, since in many cases the number of such bodies is seen to be halved in the division process, and division of each occurs later. In almost all instances however, the internal changes in the pyrenoid accompany the changes in mitosis. It is planned to make a detailed study of the behavior of the chromatophores and pyrenoids, with special reference to their relation to the mitochondria of the cell, the subject of a later paper.

Behavior of the Flagellum and History of the Kinetic Elements.

In the early prophase the external flagellum seems to become more viscous and sticky so as to adhere readily to external objects or to the oral region of the animal. Many stained specimens show the flagellum much shortened and thickened at this stage, with abundance of foreign particles adhering to it. Frequently the end adheres to the body near the cytostome and with the foreign particles attached obscures the opening into the gullet. This evidence of dissolution progresses inward, eventually involving the two branches in the reservoir, as well as the blepharoplast and basal granule on the lower border. In a few instances remains of the old flagellum have been observed in the late metaphase (Fig. 7). As a rule however, the flagellum seems to have been absorbed or sloughed off at a much earlier stage. Berliner has described a somewhat similar degeneration in *Copromonas major*. Other observers describe the disappearance of the motor apparatus during division; Wallengren (1905); Dobell (1908); Hartman and Chagas (1910). In other instances however, the flagella are said to persist for one daughter cell and new ones are developed in the other: Cutler (1919); Jameson (1914); Whitmore (1911); Awerinzew (1907).

In *Euglena agilis*, the origin of the motor apparatus can be traced to the budding off of the kinetic complex from the endosome during the early prophase. As a rule, the new flagella do not begin to grow until the nucleus has migrated anteriorly to the lower border of the reservoir, and the blepharoplasts have been produced from the kinetic mass. In a few cases what appear to be the new flagella have been observed previous to this time. The growth of both branches of each new flagellum progresses rapidly and by the time the body begins to separate into two daughter animals the main branch at least, has penetrated to the exterior.

It has been impossible to trace the origin of the lens shaped thickening on the main branch of the flagellum. It seems to arise gradually from the blepharoplast while the latter remains in connection with the kinetic mass on the nuclear membrane. When first observed during the quiescent period after division, it appears as a thick fiber extending toward the blepharoplast. Later it seems to condense at about the region of the stigma, increasing in size as the reorganization process is completed.

The significance of this thickening has not been determined. It appears to have no connection with the mitotic phenomena, but disappears and a new one is reconstructed with each division. Its association with the stigma suggests that it functions in some way as a sensitive area to perhaps both heat and light. It perhaps initiates the motor reflex in *Euglena* as described by Mast, Jennings, Bancroft and others. Steuer describes a similar thickening on both the flagella of *Eutreptia*, in approximately the same position with relation to the stigma. This may represent a mere duplication of the structure as found in *Euglena* and serve the same function for the two flagella, the one directed anteriorly and the other posteriorly.

Discussion.

The importance of the flagellates as objects of study has long been recognized by students of the Protozoa. Bütschli (1878), Klebs (1883), Fisch (1883), and many other earlier workers did much to stimulate such research. The Euglenoid nucleus has proved to be a very favorable object for study of nuclear phenomena during mitosis and yet not until comparatively recent time were the nuclear phenomena here critically described and figured. Much controversy has arisen because of superficial observation as regards both the structure of the resting nucleus and the process of nuclear division. Tschenzoff (1916), presented one of the most complete and critical studies of *Euglena viridis* recorded and attempted to interpret the nuclear phenomena. Belar likewise, gave a good account of the process in *Astasia*, although his descriptions and figures seem to be somewhat incomplete. Other workers, Rhodes and Kirby (MSS.) on *Heteronema*, Hall (1923) on *Menoidium* have given more detailed accounts. Much further study must be made before many of the disputed points can be settled.

The Resting Nucleus.

The nuclei of flagellates present varied appearances. Prowazek (1903), attempted to classify the various types and Dobell (1908) grouped these types under the following four classes, adding a fifth class himself:

(1) Simple nuclei, with an evenly distributed chromatic network, and no internal structures (karyosomes, division centers, etc.), *e.g.*, *Herpetomonas*.

(2) Vesicular nuclei, with direct division; with a central chromatin mass surrounded by a clear zone, across which a more or less distinct network extends outward to the nuclear membrane. Such a nucleus may be seen in some species of *Bodo*, and is well represented by *Copromonas*.

(3) Centronuclei containing a "nucleolo-centrosome" (Keuten, 1895) and separate chromatin masses. This type of nucleus is characteristic of *Euglena* and its allies. (The centronucleus, as defined by Boveri, is a nucleus which contains a cyto-center, either in consolidated or diffuse form. In the case of *Euglena*, etc., the cyto-center is the nucleolo-centrosome, *i.e.*, is of the consolidated type.)

(4) Vesicular nuclei, with karyokinetic division, *e.g.*, *Polytoma*, *Chlamydomonas*, etc.

(5) Nuclei in which the achromatic division center lies freely in the cell, whilst the chromatin is diffuse in the form of chromidia.

According to this system of classification, the nucleus of *Euglena agilis* is of type three, yet the fact of the apparent karyokinetic division would indicate a modification of the type.

Various descriptions have been given of the Euglenoid nucleus: Bütschli (1889), Steuer (1904), Haase (1910), Hamburger (1911), Tschenzoff (1916), Hall (1923), considered it similar to the nuclei of other plant and animal cells so far as the chromatin material was concerned. Klebs (1883), Dangeard (1902), Dehorne following Dangeard (1920), record a spireme structure in the resting nucleus. It is quite probable that the latter descriptions are of nuclei in the early prophase stages of division rather than in the resting condition.

Keuten (1895), described well formed chromosomes in the resting nucleus of *Euglena viridis*, but here again it seems probable that prophase stages were observed.

The description as given by Tschenzoff (1916), seems to be typical for many of the Euglenoid nuclei, that of Euglena agilis included. He described a central "Binnenkorper" around which is found "chromatische substanz in Form von feinsten Brockchen und Fadchen. Manchmal tritt mehr brockliche Beschaffenheit des Chromatin, manchmals feinfadige Zutage. Manchmal die Chromatin konnen auf einer Netwerk aufgereiht zu sein, . . . Ein geschlossenes Webenwerk konnte ich nicht festellen, wenn auch das Vorhandsein eines solches zu vermuten ist."

This description agrees with that of Butschli (1883–1889) for nuclei of Euglenoidina in general and with the descriptions by many other observers. It might well be added that in several species of *Euglena* examined the endosome is fragmented, the fragments often being indistinguishable from the granules of chromatin when stained with hæmatoxylin.

On the whole, there seems to be no single distinct type of nucleus characteristic of the Euglenidæ. We must face the fact therefore, that much more detailed work needs to be done on the group before an accurate classification of the nuclei can be made.

The Endosome.

This structure has led to much discussion among various observers as to both structure and function. It was first defined by Minchin as being the chromatin mass at or near the center of a "vesicular" nucleus, the precise nature of which not being determined in the definition. There may or may not occur in such a nucleus other granules of chromatin. If they do occur they are always smaller than the central mass or endosome. Some observers describe the structure as a body of homogeneous character comparable to the nucleoli of other plant and animal cells. Others say it consists of a matrix of plastin impregnated with chromatin, arranged more or less in granules, and plays a part in the formation of chromosomes (Berliner, 1909; Haase, 1910; Schüssler, 1917; Hartmann, 1919, 1921). Still others, Keuten (1895) compare it with the centrosome and central spindle of diatoms and speak of it as the "nucleolo-centrosome." Schaudinn (1896) thought he had demonstrated this to be the nature of the endosome in Oxyrrhis when by placing the animal in dilute sea-water the endosome migrated from the nucleus and functioned as an extranuclear centrosome. Hall (1924), after critical study of *Oxyrrhis*, thinks Schaudinn's supposed endosome was a small food mass. Steuer (1904) describes a "nucleolo-centrosoma" in the center of the nucleus of *Eutreptia*. In the work of Tschenzoff and in the general work of Doflein the body is spoken of as the "Binnenkörper," which term is quite non-committal both as to structure and function.

The question of the presence of a centriole in the endosome has also been the subject of much discussion. Schüssler (1917) believes that both nuclear components are located in the endosome of *Scytomonas*, and that from this type as a starting point all degrees of differentiation of the kinetic and generative components may be found. Belar (1916) figures definite centrioles in the endosome of *Astasia*, tracing them through their entire cycle. In *Rhynchomonas*, he describes a centriole which passes from the karyosome to the nuclear membrane where it divides. In the same paper (1916) he concludes that "the basal body of Flagellates is doubtless a centriole."

A good summary of the relation between the kinetic and generative components of the nucleus, bearing upon the problem of the endosome, is to be found in the work of Hartmann (1918) and of Jollos (1917):

"Denn wir haben ja eingehend gesehen, wie in dem Telophasen bei Chlorogonium elongatum zunächst aus dem gesamten Kernmaterial, den Chromosomen (der generative Komponente) wie Centriole (der Teilungskomponente) ein einheitlicher Binnenkörper, also ein primäres, echtes Caryosome gebildet wird. Erst sekundär trat das Chromosomenmaterial (sowie offenbar das Centriol) in den aussenkern über und es bleib nur ein einfacher Binnenkörper oder Nucleolus zurück, der nun weder die generative noch die Teilungskomponente enthält." Page 28, Hartmann.

"Der Binnenkörper kann vielmehr, je nachdem bei den verschiedenen Protisten oder in verschiedenen Entwicklungstadien ein und derselben Form, entweder beide Komponenten enthalten, oder die eine oder die andere, oder schliesslich überhaupt keine. Das Wesenlichte unserer Anschauung, mit den alle neuren Tatschen übereinstimmen, ist 'die Lehre, das bei der Teilung allen Protisten, der einfachsten wie der hochstenwickelten zwei Komponenten hervortreten, die lokomotorische und die idiogenerative, und dass die mannigfaltige Konstitution der Protistenkerne durch die wechselnde Anordnung und Ausbildung dieser beiden Komponenten bedingt wird.' Jollos, 1917." Hartmann, page 28.

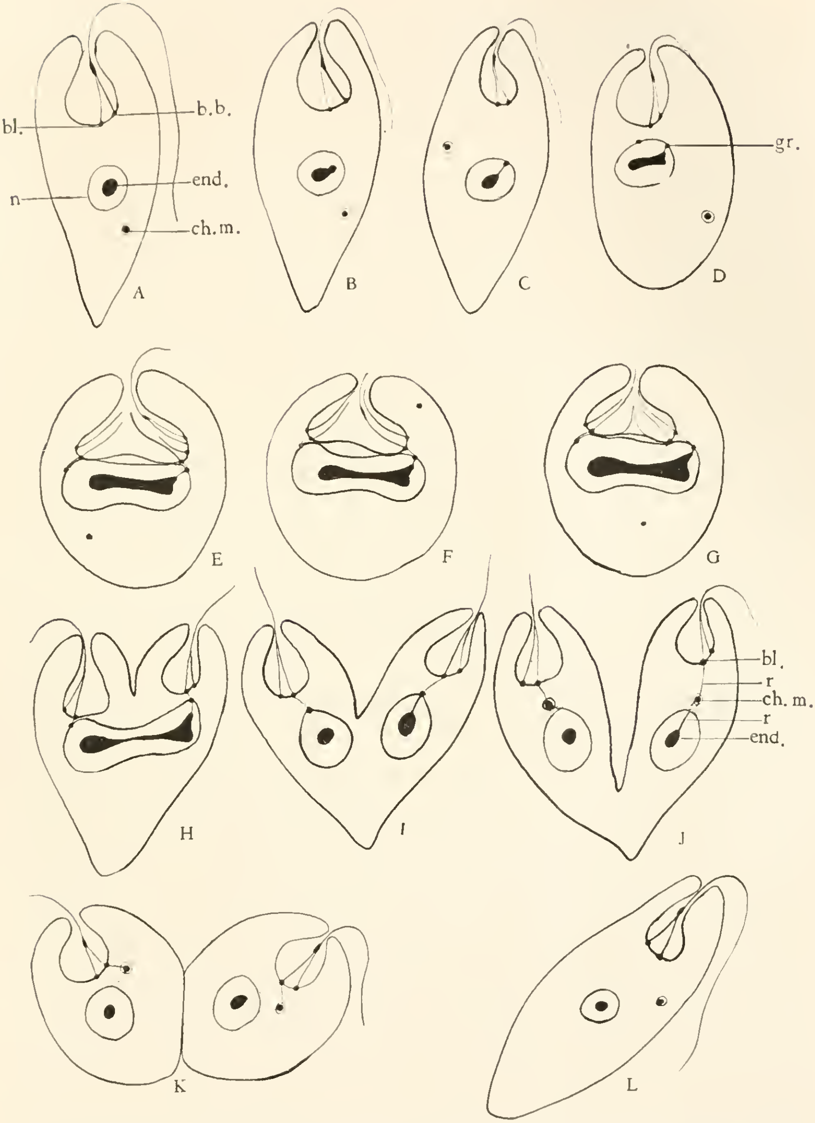
In *Euglena agilis* certain phases of this intranuclear body seem to shed some light on the endosome problem. One of the first evidences of approaching mitosis is the budding off from the endosome of a deeply staining granule of unmistakable character (Figs. 2, 3). This granule passes to the nuclear membrane where it divides, the division products taking their place at opposite sides of the nucleus, although not at the exact poles. Throughout the division process a rhizoplast may be seen connecting the original granule with the endosome. From each of the daughter granules are budded off the blepharoplast and basal body for the motor apparatus. The entire system remains integrated as a distinct unit until the division is completed and the animal goes into the quiescent period.

The portion of the endosome remaining in the nucleus, lengthens out during division, becoming first rod-shape, then spindle-shape and finally dumb-bell shape, but through all the changes in shape the opposite ends are seen to be different. One is rounded or knob-like while the other is blunt and possesses an elongation on the anterior side, from which the intra-nuclear rhizoplast may be traced to the kinetic complex. As division progresses and the centrodesmose elongates, the opposite ends of the endosome seem to be drawn upward toward the two kinetic centers, or rather the two masses from which the motor elements have been developed. The mass of the endosome appears as a passive frame work or support serving as a central spindle for the division process, and not as a kinetic center for the division.

Whitmore (1911) describes in *Prowazekia asiatica* a process quite similar to that observed in *Euglena agilis*. According to him a body is pinched off from the karyosome which has loosened up during the early stages of division. From this body arise the centrioles while the remainder of the karyosome together with the outer chromatin of the nucleus forms the spindle. He concludes that the body pinched off is the locomotor-generative component, the karyosome residue constituting the idio-chromatin.

In diphasic amebæ which combine amœboid and flagellated phases in one life cycle, *e.g.*, *Nægleria gruberi* (Wilson, 1915), a granule in the endosome gives rise to the blepharoplast by division. One daughter-granule destined to become the blepharoplast, migrates to the nuclear membrane, remaining connected with the granule in the endosome by an intra-nuclear rhizoplast. The flagellum grows out from the blepharoplast, hence the motor apparatus in these forms arises directly or indirectly as an outgrowth from the endosome. The process in *Euglena agilis* is not quite as simple as this since the mass budded from the endosome contains blepharoplasts, basal bodies, and a chromatoid residue, which remains in close connection with the motor apparatus until the daughter animals become fully reorganized after division.

McCulloch (1917) describes the origin of a chromatoid mass from the endosome in *Crithidia euryophthalmi*. This mass later is seen connected with the blepharoplast and the endosome by means of rhizoplasts. She identifies the structure as the parabasal body. A somewhat similar body is figured in *Cryptobia* by Swezy, who likewise speaks of it as the parabasal body. The resemblance between these figures and certain stages in the cycle of *Euglena agilis* is readily seen (Text-figure *A*).



TEXT-FIGURE *A*. Diagrams showing the origin and development of the motor apparatus in *Euglena agilis*. *A*, vegetative stage; *B–D*, stages in the prophase; *E*, metaphase; *F–H*, anaphase; *I–J*, telophase; *K*, cells in quiescent period; *L*, daughter animal reorganized. (*bl.*) Blepharoplast; *b. b.* basal body; *end.*, endosome; *ch. m.*, chromatoid mass left as residue after formation of kinetic elements; *n*, nucleus; *gr.*, granule which arises from the endosome, divides and gives rise to the kinetic elements; *r*, rhizoplast.

The so-called "kineto-nucleus" of trypanosomes appears in approximately the same relation to the rest of the cell as does the deeply staining chromatoid mass of the kinetic complex in *Euglena agilis*. When one remembers the importance given to the "kineto-nucleus" and the emphasis placed upon homologous structures in other forms, in the development of the group *Binucleata*, the interest attached to the comparable body in *Euglena agilis* is increased. It is generally agreed by Protozoölogists the "kineto-nucleus" is neither a nucleus nor an active kinetic center and the name should therefore be replaced by one of greater significance. Among the terms suggested is "parabasal body," first used by Janicki but redefined and used more extensively by Kofoid.

Examination of the descriptions given of parabasal bodies and their behavior by Janicki and others, shows striking similarity to that of the chromatoid mass of the kinetic complex in *E. agilis*. The parabasal body is closely connected with the kinetic elements of the cell, and according to Kofoid serves as a reservoir of substances which are used by the organism in its kinetic activities. During mitosis the body behaves variously in different species. In *Devescovina striata*, for example, it divides and the two daughter parabasals are shown in constant relationship to the poles of the extra-nuclear, rod-like, division spindle, where centrioles can often be determined. In other cases, *e.g.*, *Lophomonas blattarum*, the parabasal body degenerates with approaching mitosis and a new one is formed for each of the daughter animals produced. In *Euglena agilis*, the granule which migrates from the endosome, divides, the daughter halves separate and occupy positions at the approximate poles of the division figure. From each granule is freed a blepharoplast-basal body complex which becomes the base of the bifurcated flagellum. A chromatoid residue remains on the nuclear membrane, connected by a rhizoplast to the blepharoplast and in one animal to the endosome. The residue retains this intimate connection with the motor apparatus until the animals are completely reorganized, at which time it breaks away from the nuclear membrane, the rhizoplasts disappear and it passes into the cytoplasm. As the next mitosis approaches, it begins to degenerate as a rule, and finally disappears entirely.

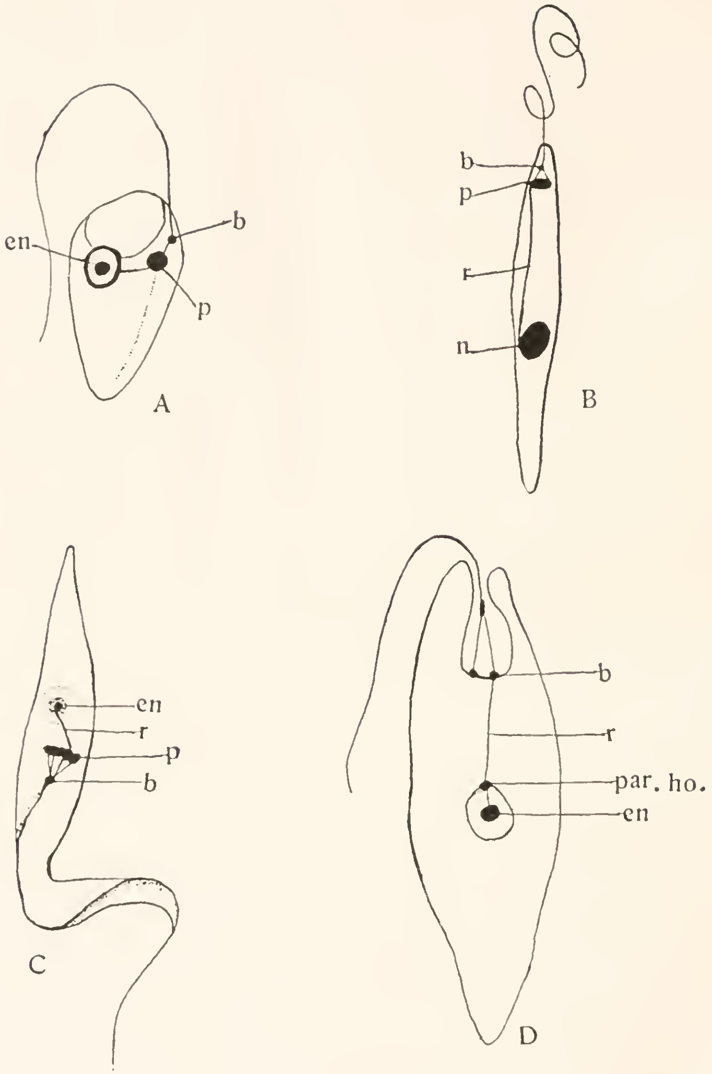
Such a type of organization and behavior of related and kinetic elements is what one might expect in so primitive an organism as *Euglena*. We have many valid reasons for believing that within this group are to be found the progenitors of the other groups of Protozoa. As evolution progressed toward the rhizopod type, exflagellation might easily be conceived as a process of inhibition of the development of the kinetic complex from the endosome of the nucleus. When conditions in the cycle of an amœba, *e.g.*, *Nagleria gruberi*, are appropriate, flagellation is stimulated and we see the complex arising from the endosome as in *Euglena*.

In those flagellates which have a more diverse series of life habits and hence greater complexity of structure, such as Trypanosomes, *Devescovina*, *Crithidia*, *Lophomonas*, *Calonympha*, etc., we find a type of organization easily derived from some stage shown in the development of *Euglena*. In these parasitic flagellates which show the presence of a permanent parabasal body in connection with the motor apparatus, we can easily conceive that the chromatoid residue which remains in connection with the motor apparatus in *Euglena agilis*, only during the late division stages and the reorganization period, becomes a permanent organelle of the cell. Such a change might be brought about through the greater demands made upon the motor apparatus when the organisms become adapted to a dense medium. In *E. agilis* the residue serves as a temporary reserve only, for the kinetic elements of the cell and becomes disconnected from them as soon as the new flagellum begins to function actively. A permanent reservoir of substances to be used by the organisms in its kinetic activities is required by the parasitic flagellates. This need is met by the parabasal body.

We may draw the following conclusions from the preceding discussion:

(1) The endosome in *Euglena agilis* is the ultimate source of all the kinetic elements of the cell, but does not contribute to the formation of the chromosomes.

(2) An endobasal body is located in the endosome and gives rise by division to a chromatoid mass, the kinetic complex. This migrates to the periphery of the nucleus where it divides, the daughter halves passing to the approximate poles of the division



Text-figure *B.* Diagrams showing the suggested homology between the kinetic elements of *Euglena agilis* and other flagellates. *A*, *Cryptobia* sp.; *B. Herpetomonas musca-domesticae; C, Trypanosoma cruzi; D, Euglena agilis.* (*b*) Blepharoplast; (*r*) rhizoplast; (*p*) parabasal body; (*en*) endosome; (*n*) nucleus; (*par. ho.*) parabasal homologue. (*A* and *B* from Calkins after Swezy;) *C* from Calkins after McCulloch.)

figure. In this position the bodies seem to be homologous to the centro-blepharoplasts of Trichomonas and other forms as described by Kofoid.

(3) The chromatoid residue left on the nuclear membrane after the blepharoplast-basal body complex is freed, as described above, is homologous to the parabasal body of many parasitic flagellates as well as to the so-called "kinetonucleus" of Trypanosomes. This homology is indicated by the position of the residue with relation to the poles of the division figure and its connections with the endosome and blepharoplast.

Examination of Text-Fig. *B* indicates the homologies suggested.

Mitosis.

Many cases of "amitosis" were described by the earlier observers of division stages in the Protozoa. More refined methods of technique have enabled later workers to determine, however, that amitosis is of rare occurrence in this group as it is in Metazoa. Nagler (1909) designated what had been previously called "amitosis" in amœba and many other Protozoa, as "Promitosis," in order to differentiate this type from mitosis as found in Metazoa and Metaphyta. He says: "Für die sogenannte Amitose der Protozoen führt man daher am besten eine neue Bezeichnung ein und definiert sie als eine Kernteilung, die weder ausgesprochene Mitose, noch Amitose ist und sich charakterisiert durch die Teilung eines Nucleolocentrosoms, des Caryosoms. Ich schlage deshalb für diese Teilungsform den Namen Promitose vor."

Chatton (1910) distinguished three types of mitosis, characterized as follows:

(1) Promitosis—Division center within the karyosome, nuclear membrane persistent, chromosomes formed from the peripheral chromatin.

(2) Mesomitosis—Division center outside the karyosome, nuclear membrane persistent, chromosomes from the karyosome.

(3) Metamitosis—Division center outside the nucleus, nuclear membrane disappears, chromosomes from the nuclear chromatin.

This system is based on a study of *Amœba* and hence could scarcely be expected to apply to other forms equally as well. *Euglena agilis* seems at first sight to fit into type one or promitosis, and yet the division center cannot be definitely located within the endosome.

Alexeieff (1913) elaborates a system of classification which goes into great detail, but which even so, does not cover all cases. According to his system, *Euglena agilis* seems to show haplomitosis, since no centriole can be positively identified and yet distinct polar masses are formed.

Prophase.—The formation of chromosomes in *Euglena agilis* does not occur as was described by Tschenzoff for *E. viridis*. In the resting stage the chromatin is arranged in masses, frequently dumb-bell shaped, along an achromatic network. In the early prophase these separate masses appear to enlarge and increase in staining capacity, at the same time fusing along the linin threads of the network to form chromosomes. Tschenzoff describes the formation of a number of granules around the periphery of the nucleus which eventually become arranged in rows or files. These become the chromosomes. It is an easy matter to make out granules around the periphery of the nucleus in *E. agilis* at this stage, but upon close examination they prove to be optical cross sections of chromosomes. Also, the granules of the motor complex may be found in a similar position, staining with the same intensity as does the endosome.

I have not been able to make out the beaded structure of the chromosomes, such as have been figured by some investigators. It is true that each chromosome appears to consist of two masses in its earlier stages of formation and later these masses seem to run together to form the smooth chromosome. It is difficult to interpret this apparent double structure of the chromosomes. It is possible that it represents a doubling or splitting at some previous stage in the cycle. Wenrich describes such an appearance of chromosomes in *Trichomonas* and traces it back to a constriction in the previous anaphase. He does not connect this constriction with the split chromosomes of the prophase however, nor does he attach any particular importance to the phenomenon.

Kofoid describes a pseudo-reduction of chromosomes in

Trichonympha, brought about by a pairing of single chromosomes in the prophase. He suggests the possibility of a pregametic doubling of chromosomes in Protozoa such as occurs in the Metazoa, but does not consider this to be the most plausible explanation of the observed facts.

Stevens describes the association of chromosomes in pairs during the vegetative stages of *Boveria subcylindrica*. The significance of this apparent reduction in vegetative mitosis is problematical and the subject requires much more work before a correct interpretation and correlation can be made. In *Uroleptus mobilis* Calkins finds eight chromosomes which unite in four pairs during the first maturation division, the pairs splitting at the second maturation division so that the daughter nuclei have four single chromosomes. He concludes that this is undoubted synapsis and reduction.

Tschenzoff describes a telophasic split in *Euglena viridis*, which doubles the number of chromosomes entering the resting vegetative stage. Such a precocious split has not been observed in *Euglena agilis*, although the double appearance of the chromatin masses may be indicative of such a process. On the other hand, a definite longitudinal split has been observed in the prophase.

It is not known what changes take place in the nucleus during the encystment in *Euglena*, but it is entirely possible that some phenomena will be found here to throw light on the apparently paired arrangement of the chromatin masses.

Metaphase.—Tschenzoff describes in the metaphase, a separation of daughter chromosomes formed by a splitting in the telophase of the preceding generation. He states that the elements may be seen paired on the spindle at this stage and then separating without further split. Structures may be observed in *Euglena agilis* which might easily be interpreted in this manner but which show on critical examination an entirely different situation. The network of chromatin in the resting nucleus becomes enlarged at the nodes or at the points where the chromatin masses are fusing to form chromosomes. This increase in bulk of chromatin does not seem to be compensated for by a corresponding increase in size of the nucleus. Since the nuclear membrane remains intact during the entire process of division the chromosomes, as soon as formed, become quite tortuous in the limited space. The enlarging endosome with the budding off of the kinetic complex tends to force the chromosomes toward the periphery of the nucleus thus forming many twists and loops. When these chromosomes come to lie radially around the elongated endosome, preceding the longitudinal splitting, they appear as loops whose arms are rather closely pressed together. Thus at one focus one can distinguish a number of pairs of distinct chromosomes, but as the focus is changed the ends may be found to be connected into the loop. The longitudinal split begins at the end next to the endosome and proceeds toward the periphery. Thus, if an apex is inward, as is frequently the case, a double V-shaped figure is produced as the split halves separate. An X shape or a figure 8 shape is produced if the free ends are turned inward. In either case, the pulling apart of the split halves forms the equatorial plate figure, or as it might be better expressed, the "equatorial cylinder."

Anaphase.—From the equatorial cylinder stage the ends of the split chromosomes pull apart and an appearance of a transverse splitting of chromosomes is the result. This perhaps accounts for some of the earlier descriptions and widely quoted figures which show a transvers splitting of chromosomes in *Euglena*.

In the late anaphase, the chromosomes begin to show evidence of a return to the typical vegetative condition. Dehorne (1920) describes them as going back into the continuous spireme stage of the resting nucleus. It is true that the exact identity of the chromosomes tends to be lost as they begin to condense into the granular condition, but little evidence is found that a continuous spireme is constructed.

Telophase.—Careful search was made for a precocious splitting at this stage in order to determine whether the description as given by Tschenzoff for *Euglena viridis* held also for *Euglena agilis*. Aside from the apparently diffuse nature of the chromosomes and their change in intensity of staining reaction, no definite evidence of a split was observed.

The Residual Mass from the Kinetic Complex.

Blochmann (1894) found in *Euglena velata* a body in the cytoplasm, often lying near the nucleus but sometimes far from it.

He first regarded it as a centrosome but because of its appearance in different parts of the cell, and because it appeared to persist after division was completed, he did not commit himself to this interpretation.

Keuten (1895) likewise found bodies lying near the nucleus during the division of *Euglena viridis*, which persisted after division and could be found surrounded by a halo in various parts of the cell in the vegetative condition. He did not think these were centrosomes nor in any way related to the division phenomena.

Haase (1910), in *Euglena sanguinea*, traces the flagellum from the lower border of the reservoir into the cytoplasm where she finds and figures both branches ending in a basal body (diplosome?).

Tschenzoff (1916) describes granules in the cytoplasm of *Euglena viridis* but explains them as artifacts resulting from his methods of killing.

One is led to believe from the above descriptions that the granule observed in the different species mentioned is the same as that traced through its entire development in *Euglena agilis*. It has been seen to arise from the endosome, pass to the periphery of the nucleus, divide into two equal daughter granules which pass to opposite of the nucleus. In this position the granules seem to be homologous to the centro-blepharoplasts as described by Kofoid. In the early prophase stages each granule produces a blepharoplast which passes to the border of the reservoir and later gives rise to the main branch of the flagellum. The chromatoid residue left on the nuclear membrane increases in size during the telophase, and during the quiescent period when the nucleus migrates posteriorly to its original position in the cell, breaks away from the nuclear membrane, becomes surrounded by a clear hyaline vesicle and becomes a cytoplasmic structure. During the vegetative stages no change can be observed in this granule, but as the stages of the next successive mitosis proceed it is seen to lose its staining capacity and as a rule disappears before the late anaphase. In some cases it has been seen to persist throughout the division and to be distributed to one of the daughter animals. In this case a single cell would be seen to contain two such granules during the early stages after division. This would account for the fact that Blochmann records three instances where he found two granules in the body of an animal. In one case he records three as being present.

The question arises as to the significance of this bit of nuclear material sent into the cytoplasm at each division period. It seems clearly demonstrated that it acts as the vehicle for the transfer of the kinetic elements from the endosome to the cytoplasm. After the kinetic elements are freed it remains in close connection with both the endosome and the blepharoplast for sometime after division, in fact until the daughter cells are fully reorganized. In this position we may conclude that it is homologous, both in structure and function, to the parabasal body of parasitic flagellates. It seems to serve as a reserve of kinetic energy until the newly developed motor apparatus is fully formed and functioning normally. With this function completed, does it play any further rôle in the physiology of the cell? Only conjecture can be made at present. It seems reasonable to suppose however, that there is sufficient chromatin material in the mass to bring about some sort of reorganization in the cytoplasm of the body after each division. Thus the protoplasm might be kept in a proper state of physiological differentiation so as to make process of conjugation, autogamy or endomixis unnecessary. The disappearance of the mass in the early stages of each division is suggestive that in *Euglena* we find a mechanism which functions as a sort of regulator of the division cycle. It is possible that changes in this mass of chromatin, this kinetic reserve, may be the stimulus which incites the division of the cell with such striking regularity. Although it does not function as a division center, yet it sets up a series of concatenated events which result in the final production of two fully vigorous individuals from the single parent cell.

Summary and Conclusions.

1. Mitosis in *Euglena agilis* is accomplished in a manner quite similar to that described for higher plants and animals. Definite chromosomes are formed, divide longitudinally and are distributed equally to the daughter cells. The chromosomes are

formed from the chromatin of the outer nucleus and not from the endosome.

2. Division of the nucleus is preceded by its migration anteriorly in the body until it comes into close contact with the lower border of the reservoir.

3. A chromatoid mass, the kinetic complex, originates from the endosome during the prophase, migrates to the periphery of the nucleus where it divides. The division products pass to opposite sides of the anterior border of the nucleus, in which position they suggest homology with the centroblepharoplasts of *Trichomonas*. No paradesmose has been detected between them however.

4. From the kinetic complex a blepharoplast is freed and produces the main branch of a new flagellum. Later, a basal body arises from each blepharoplast, and from it is produced the secondary branch or anchoring root of a flagellum.

5. The residue from the kinetic complex, after the formation of the blepharoplast, remains on the nuclear membrane until division of the animal is completed and the daughter cells have reorganized. A rhizoplast passes from it to the blepharoplast and in one of the daughter animals an intra-nuclear rhizoplast is seen making connection with the endosome. After reorganization of the daughter animals is completed, the residual mass is cast out into the cytoplasm where it remains during the vegetative stages of the organism as a deeply staining granule surrounded by a lightly staining halo. It usually disappears during the early phases of the next successive mitosis. Its behavior and relationship to the motor apparatus suggests a homology with the parabasal body of parasitic flagellates.

6. The division process is a function of the cell as a whole, being initiated not by the division of a specific body that might be called a centrosome, but rather by simultaneous changes in all parts of the cell. The centroblepharoplast homologue, together with an endobasal body in the endosome seem to constitute the principal dynamic centers. The mass of the endosome seems to constitute a sort of passive framework or spindle for the division process.

7. The entire motor apparatus of the parent animal, consisting of flagellum, basal body and blepharoplast disappears during division and a new one is built up in each daughter animal.

8. The mitotic and related phenomena in *Euglena agilis* give additional evidence of the primitive character of the Phytomastigoda. The development of the motor organoids indicate relationship with diphasic amœba on the one hand and with the complex parasitic flagellates on the other.

9. Changes in the chromatophores during the division and vegetative periods, as well as their changes when the *Euglenæ* are subjected to different cultural conditions indicate that these structures are not satisfactory criteria upon which to base the classification of the group.

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EXPLANATION OF FIGURES.

Plate I.

All sketches were made with camera lucida with Leitz compens ocular (20) and Leitz apo. obj. 1.5. They have been reduced one half.

Fig. 1. *Euglena agilis* in vegetative condition showing nucleus, endosome, chromatophores with pyrenoids, blepharoplast, basal body, flagellar enlargement, reservoir, gullet or cytopharynx, and kinetic residue. The stigma or eyespot does not show with the stains used. The chromatin is arranged in a net work.

Fig. 2. Early prophase drawn in optical section showing first stages in formation of chromosomes and early budding of the kinetic complex from the endosome.

Fig. 3. The chromosomes appear as elongated rods or loops and give the appearance of a spireme. The bud from the endosome is definitely connected by a rhizoplast.

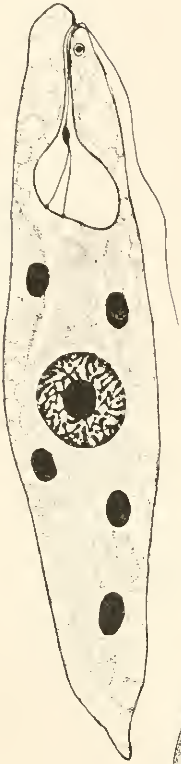
Fig. 4. The granule budded from the endosome has divided, the daughter halves passing along the periphery of the nucleus. One is connected by rhizoplast to the endosome.

Fig. 5. Another view of the daughter granules separating on the nuclear membrane. The animal is beginning to round up and the nucleus is approaching lower border of the reservoir.

Fig. 6. The endosome has elongated, each daughter half of the kinetic complex has given rise to a blepharoplast and this in turn to a basal body. From both the blepharoplast and basal body a flagellar element has grown out. The remainder of the old flagellum is seen in the right side of the figure. The pyrenoids of the chromatophous are undergoing change in organization. The chromosomes are arranged around the elongated endosome.

Fig. 7. An optical section of a late prophase or metaphase with chromosomes arranged somewhat radially around the endosome. Each chromosome shows a longitudinal split and the looped nature of each gives the appearance of a pairing.

Figs. 8–10. Stages in the anaphase, showing separation of the daughter chromosomes and the formation of an equatorial cylinder. The rhizoplast connecting the blepharoplast to the residue of the kinetic complex is visible in figure eight.



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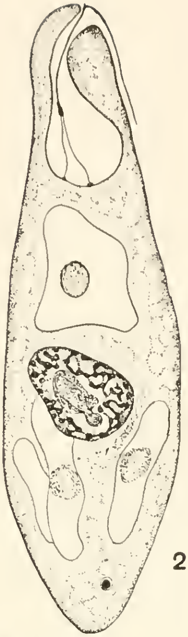

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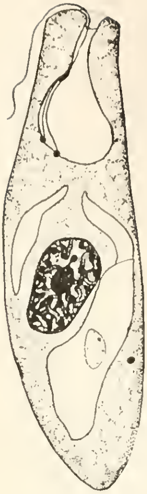

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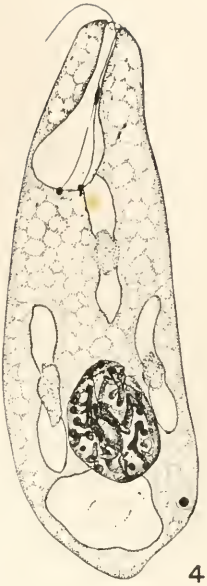

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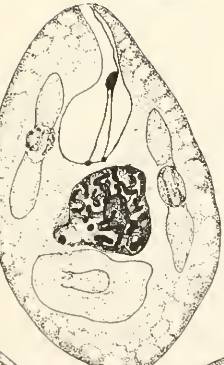

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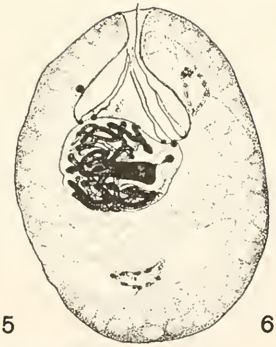

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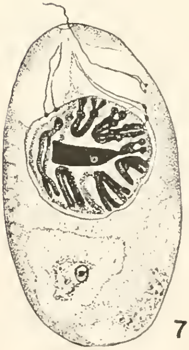

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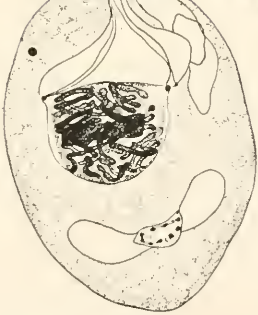

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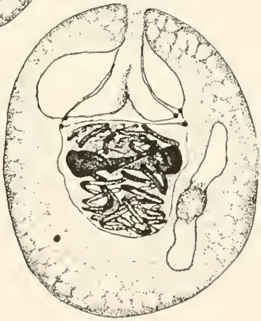

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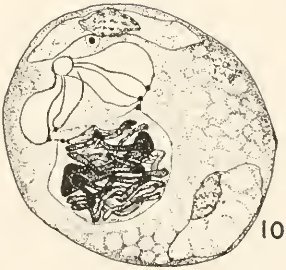

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PLATE II.

FIGS. 11–12. Late anaphases showing chromosomes grouped around ends of elongated endosome. In Fig. 11 the integrated kinetic elements show on both sides of the nucleus while they are visible on one side only in Fig. 12. The components of the other side are at a much lower level.

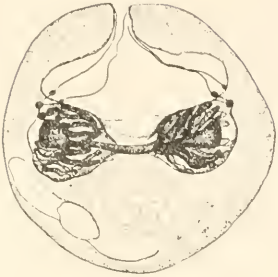
FIGS. 13–14. Telophase stages showing enlargement of residue from kinetic complex and beginning of formation of halo around it.

FIG. 15. Division completed and the daughter organisms in quiescent period. The nucleus is receding from the reservoir.

FIG. 16. The chromosomes have begun to go back into vegetative condition. The kinetic residue is cast into cytoplasm.

FIG. 17. Further stage in reorganization.

FIG. 18. Organism reorganized.



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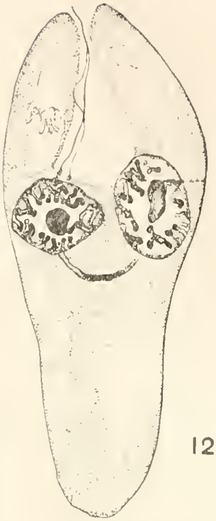

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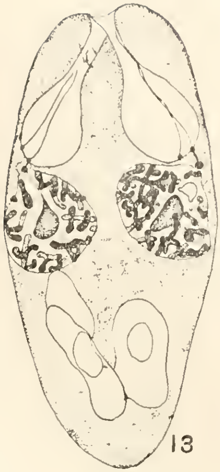

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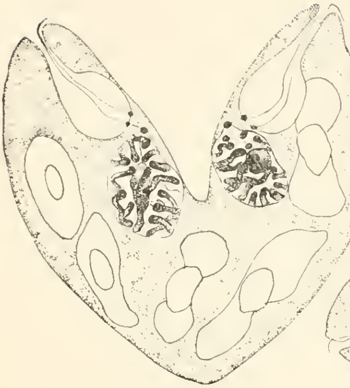

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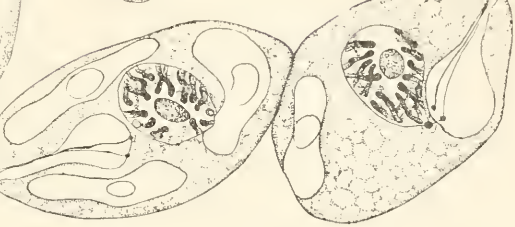

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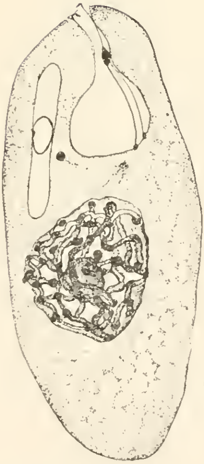

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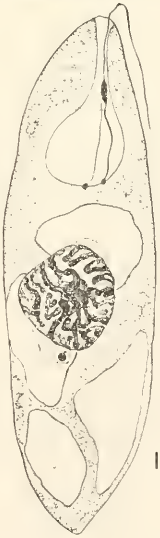

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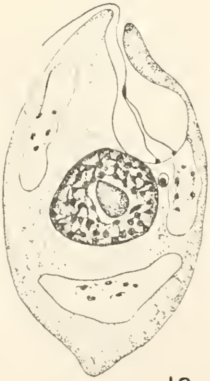

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WOOLFORD B. BAKER.

HYDROGEN ION CONCENTRATION IN THE BLOOD OF CERTAIN INSECTS (ORTHOPTERA).

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Inasmuch as most accurate quantitative results on the hydrogen ion concentration of blood and body fluids have been largely confined to higher animals it seems highly desirable to extend these observations to lower forms, and especially insects. Since only small amounts of fluids are usually available from single insects, an accurate micro-electrometric method is necessary for the successful carrying out of such investigations. A simple micro hydrogen electrode and vessel, particularly designed for this purpose, have been described by the author (Bodine and Fink, 1). The present paper contains data obtained by the use of this method for the blood of different species of grasshoppers.

With the exception of two papers (Bishop, 2; Brecher, 3), all previous work on insect blood seems to have been carried out by means of colorimetric methods (Jameson and Atkins, 4; Crozier, 5; Bodine, 6; Glaser, 7). Since the blood of most insects is colored it seems highly desirable that other methods than micro-colorimetric ones be used and especially since the indicator most used in these methods is brom-thymol-blue. The blue-green to yellow-green color of insect blood seems to introduce an undesirable factor in micro-colorimetric methods using this indicator. Results obtained by the use of colorimetric methods by different authors are somewhat variable and it therefore seemed highly desirable to check them by means of an electrometric method.

In the present investigation different species of grasshoppers, reared under controlled food, temperature and other laboratory conditions, have been used as well as other animals taken from their normal out-of-door environments. Individual studies have been made on the blood of certain species from the day of hatching until death or in other words during their entire life cycle. Blood was taken from the grasshoppers in a variety of ways,—by cutting off the tip of the feet, puncturing the animal by means of needles, insertion of electrode vessel directly into the body cavity, etc. No marked differences in pH of the blood obtained by these different methods was noted. The method found most satisfactory and least harmful to the grasshopper, however, was a small needle puncture into the mid-lateral abdominal wall. Only a very small amount of blood is thus lost by the insect and the wound is quickly sealed. Sufficient blood (approximately 0.01 cc.), however, is obtained in this way for electrometric determinations of pH values. The grasshopper was usually punctured and the blood immediately drawn up into the electrode vessel with practically no exposure to air. As a matter of fact, identical results were obtained with blood taken from the interior of the animal by the insertion of the electrode vessel directly into the animal as by the method of puncturing and then immediately

TABLE I.

SPECIES: *Chortophaga viridifasciata.*

Age, Color, Sex.	pH of Blood.
nymph—brown—♂	6.83
nymph—green—♀	6.83
nymph—brown—♀	6.84
nymph—brown—♀	6.86
adult—♂	6.62
adult—♂	6.43
adult—♂	6.67
adult—♂	7.05
adult—♂	6.85
adult—♀	6.65
adult—♀	6.94
adult—♀	6.51
adult—♀	6.95
adult—♀	6.73
adult—♀	6.93
Average	6.79
Range	6.43–7.05

Explanation of Table 1.

Data taken at random from many individuals showing pH of blood of *Chortophaga viridifasciata.* Animals all taken from out-of-doors. Some bloods tested at once. Other animals kept in laboratory and fed lettuce over different periods. Samples of blood tested regularly. Average given for all individuals tested.

TABLE 2.

SPECIES: *Melanoplus femur rubrum.*

Age, Sex.	pH of Blood.
Nymph	7.00
Nymph	6.23
Nymph	6.95
Nymph	6.36
Nymph	6.70
Nymph	6.59
Nymph	6.71
Nymph	7.07
Nymph	6.79
Nymph	6.96
Nymph	6.55
Nymph	6.70
Nymph	6.45
Nymph	6.66
Adult—♂	6.86
Adult—♂	6.86
Adult—♂	6.58
Adult—♂	6.80
Adult—♀	7.00
Adult—♀	6.73
Average	6.70
Range	6.23–7.11

Explanation of Table 2.

Data selected showing range of pH for blood of different individuals. All animals laboratory raised and fed lettuce. Average and range of pH for all individuals tested.

TABLE 3.

SPECIES: *Melanoplus differentialis.*

Age.	pH of Blood.
Nymph	6.85
Nymph	6.43
Nymph	6.52
Nymph	6.56
Nymph	6.50
Nymph	6.75
Nymph	6.98
Nymph	6.93
Average	6.68
Range	6.42–6.98

Explanation of Table 3.

Data selected from several hundred individuals showing range of pH values. Adult values are similar to those for nymphs. Animals reared in laboratory and fed lettuce. Average and range of pH for all individuals tested.

drawing the blood up into the vessel. Individual adult grasshoppers can be punctured at least two to three times daily without any apparent injury.

TABLE 4.

Romalea microptera.[1]

Date.	Stage.	Average pH of Blood.
Feb. 19, 1926	Nymphs	6.83
" 26, 1926	Nymphs	6.88
Mar. 5, 1926	Nymphs	6.72
" 12, 1926	Nymphs	6.74
" 19, 1926	Nymphs	6.84
" 26, 1926	Nymphs	6.79
Apr. 9, 1926	Nymphs	6.66
" 16, 1926	Nymphs	6.87
" 23, 1926	Nymphs	6.56
" 28, 1926	Nymphs	7.10 (?)
" 30, 1926	Adults	6.82
May 7, 1926	Adults	6.88
" 14, 1926	Adults	6.84
" 21, 1926	Adults	6.68
" 28, 1926	Adults	6.46
June 1, 1926	Adults	6.77
" 2, 1926	Adults	6.67
" 3, 1926	Adults	6.55
" 4, 1926	Adults	6.53
" 5, 1926	Adults	6.72
" 7, 1926	Adults	6.50
" 9, 1926	Adults	6.59
" 11, 1926	Adults	6.82
" 15, 1926	Adults	6.87
Average		6.73
Range		6.4–6.8

Explanation of Table 4.

Detailed average pH values for grasshoppers from day of hatching until death. Data for each day represents average readings for from 5 to 20 individuals. All animals laboratory raised and fed lettuce. Average and range of pH given for all individuals tested.

All laboratory animals were hatched from eggs laid in the laboratory and were fed lettuce. Animals were also collected from the outside and comparisons made with those reared entirely under laboratory conditions.

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[1] The author is indebted to Dr. H. Fry for his generosity in supplying some of the eggs used in these experiments. These eggs were from a cross between *Romalea microptera* and *Romalea microptera*, variety *macri*. No differences in pH of the blood from this cross and the *R. microptera* were noted.

Temperature during the course of the experiments ranged from 22 to 24° C.

Table 5.

Temp. 24.° C.—June 15, 1926.

	Samples—E.M.F.	Average pH.
Buffer (5.65)	(*a*) .57573	5.61
	(*b*) .57580	
	(*c*) .57560	
Romalea adult	(*a*) .65250	6.90
	(*b*) .65555	
Buffer (5.65)	(*a*) .58210	5.69
	(*b*) .58088	
	(*c*) .58006	
Romalea adult	(*a*) .64553	6.79
	(*b*) .64553	
Buffer (5.65)	(*a*) .58173	5.67
	(*b*) .57953	
Romalea adult	(*a*) .65203	6.90
	(*b*) .65273	
Buffer (5.65)	(*a*) .58265	5.67
	(*b*) .57963	
	(*c*) .57963	
Romalea adult	(*a*) .65226	6.89
	(*b*) .65163	
Buffer (5.65)	(*a*) .58200	5.67
	(*b*) .57742	

Explanation of Table 5.

Part of daily record for June 15, 1926, to show method of procedure in making determinations of blood pH values. Note that standard or buffer solution is always read before and after every reading on blood as check on electrode.

The results of the determinations are summarized in Tables 1–5. From an examination of these it is evident that considerable variation exists in the hydrogen ion concentration of the blood of the same and different species. No marked species, sex or age differences have been detected. The variations shown can hardly be due primarily to differences in food conditions since the laboratory animals were all fed the same food, lettuce, and the out-of-door animals had varied diets depending on the locality from which they were taken. The data in the tables are summarized and do not show individual variations which are to a degree closely similar to those indicated for groups. Readings for the same individual, however, are quite constant for any one day. An explanation for these consistent variations is not at hand.

It is of some interest to compare the present results with those obtained with colorimetric methods by Glaser (7), for grasshoppers (*Melanoplus differentialis*). This author found the range of pH values for the blood of these grasshoppers to be 7.2 to 7.6. In previous experiments (6), using a colorimetric method the average pH for the blood of many species of grasshoppers was found to be 6.8. It seems to the author that the results of Glaser (7) are too high, due doubtless to difficulties in the colorimetric method already indicated above.

Preliminary experiments on the CO_2 tension of grasshopper blood indicate a rather low value, which seems consistent with an acid pH for the blood and the mechanism of tracheal respiration.

Grasshopper blood seems to be slightly buffered, since upon dilution no marked changes in pH are detected until a dilution of two to four times is reached. Most of the buffer effects are apparently due to the fairly large amounts of phosphates which the blood contains. In contrast to mammalian blood there seems to be present rather small amounts of carbonates.

Results for pH values of blood for other insects obtained by means of electrometric methods (Bishop, 2; Brecher 3) 6.8, agree rather closely with those obtained in the present work for grasshopper blood.

SUMMARY.

The pH values for the blood of different species of grasshoppers have been determined by a micro-electrometric method. Considerable variation exists in the pH values for blood of the same species of animals. No correlations seem to exist between blood pH and age or sex. The range and average pH values for the blood of the different species of grasshoppers examined are as follows:

Species.	Range of pH.	Average pH.
Melanoplus femur rubrum	6.4–6.8	6.73
Melanoplus differentialis	6.42–6.98	6.68
Chortophaga viridifasciata	6.43–7.05	6.79
Romalea microptera	6.4–6.8	6.73

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Vol. LI *December, 1926* *No. 6*

BIOLOGICAL BULLETIN

INFLUENCE OF AGE OF MOTHER ON APPEARANCE OF AN HEREDITARY VARIATION IN *HABROBRACON.*

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Contentions about the inheritance of acquired characters have frequently involved questions of the age of the parents. Thus in trotting horses older sires or dams which have undergone considerable training have been supposed to transmit some of the benefits of this training to their offspring. Similarly, acquired knowledge, acquired immunity to disease, etc., have been stated to be transmitted. When these matters are investigated scientifically it is found that unintentional selection of parents may account for the bulk of cases. Nor is character of offspring which may be found to be correlated with parental age to be taken as proof of change in germ plasm of parents. Some non-germinal influence may be transmitted. Thus it has been shown (Hart, 1923) that Mongolian idiots are at their birth twenty-three times as likely to have mothers forty years of age or over as mothers from twenty to twenty-four. There is no good evidence that Mongolian idiocy is hereditary.

The material considered in the present paper presents clear evidence of change in average traits of offspring according to age of mothers. Discussion as to whether this difference is germinal will be reserved for a later part of the paper.

In the parasitic wasp *Habrobracon juglandis* (Ashmead) which has been bred under constant environmental conditions, abnormalities in antennæ, external genitalia or posterior part of the digestive tract occur.

Abnormal antennæ may be branched, bent or clubbed.

Gonapophyses of the female (sensory appendages forming sheath for the sting) may be swollen at the end or twisted from their normal position; extra gonapophyses may appear in addition to the two normally present.

These abnormalities which consist in increase in size or number of parts or in malformation without reduction are to be distinguished from *deficiency*.

DEFICIENCY.

Antennal deficiency is usually evidenced by tapering and fusion of terminal joints as in right antenna of specimen shown in Plate 1, Fig. *H* (Male from 1171.3*c*). There is, in general, a tendency toward symmetry but much irregularity. Fusion without tapering may occur or one antenna may be normal (Figs. *A* and *H*).

Males may have genitalia missing and abdomen rounded off at tip, or the normal parts may be reduced in size, twisted out of shape or lacking on one side of the body while present in almost normal condition on the other.

In Plate I., Fig. *D* is shown the ventral side of the abdomen of a normal male. Fig. *E* shows the abdomen from posterior view, somewhat oblique, with genitalia extruded. The tip is curved ventrally so that it is seen in dorsal aspect.

Figs. *A*, *B*, and *C* are from a male (Freak 60) illustrating deficiency in antennæ and genitalia on the right side of the body both anteriorly and posteriorly. Fig. *B* shows the tip of the abdomen ventrally and slightly oblique while Fig. *C* is an aspect comparable with Fig. *E*. Genitalia are completely lacking on right side, including even the right side of the penis, while posterior sternites and tergites taper off dextrally. Organs on the left are somewhat bent toward the right but otherwise normal. Sections of abdomen showed right testis and vas deferens completely lacking, left apparently normal.

Fig. *A*, ventral view of head, shows left antenna normal. It is drawn displaced from the head for economy of space. Right antenna is shortened by reduction and fusion of joints toward the tip. Right labial palpus is missing.

Figs. *K*, *L*, and *M*, show dorsal, ventral, and right aspect respectively of abdomen of normal female.

Figs. *I* and *J* show ventral and right aspect respectively of abdomen of a deficient female (Freak 6). Great reduction and fusion of parts posteriorly, especially of gonapophyses, is to be noted. Sections show large eggs and nurse cells developing, but no trace of poison apparatus; deficiency therefore extended internally to posterior organs.

Reduction in the posterior part of the digestive tract also occurs. This may be indicated by a swollen appearance of the abdomen with an abnormally dark color, due to failure of elimination of fecal matter. Sections show different degrees of defect ranging from an apparent failure of the proctenteron to connect with the mid-gut, to a complete disappearance of the former and considerable reduction of the latter posteriorly. This reduction may shorten the Malpighian tubules until they are little knobs on the posterior end of the mid-gut sack, or the latter may be rounded off smoothly. Fig. *F* shows a specimen of this type from a female (Freak 21) with no external genitalia. There were no hind-gut, no Malpighian tubules, no poison apparatus. In the ovaries nurse cells were present but ova were small. The posterior compound nerve ganglion was normal.

Fig. *G* is from a male (Freak 20) with no external genitalia, and no hind-gut posterior to the Malpighian tubules which are short and thick. The mid-gut is much swollen with fecal matter. Testes were present, filled with mature sperm but there were no vasa deferentia. The posterior compound nerve ganglion was separated into its three elements.

Rare Occurrence of Abnormalities in Normal Stocks.

Among 53,148 males and 16,036 females of normal stocks the following abnormalities in antennæ, genitalia, and digestive tract have appeared.

Only twelve females had abnormal gonapophyses.

Abnormal antennæ were recorded in only thirty males and twenty-six females. These may be classified as having one or more antennæ short, sixteen; absent, twenty-eight; clubbed, one; branched, three; and unclassified, eight. Two of those having short antennæ were males with deficient genitalia.

Association between deficiency in genitalia and digestive tract is much closer. Males deficient in genitalia alone were twenty-four; in digestive tract alone, fifty-five; and in genitalia and digestive tract, fifteen. Females deficient in genitalia alone were nine; in digestive tract alone, twenty-two; and in genitalia and digestive tract, nine.

These figures illustrate the rare occurrence of these abnormalities in normal stocks.

Deficiencies in genitalia necessarily entail sterility. Mating reactions are in all cases quite normal. A male having no trace of external genitalia and internal much reduced will mount a female and beat its antennæ in the usual manner. Females with reduced genitalia or abdomen swollen and blackened by fecal matter show characteristic responses to caterpillars. Although wasps with deficient digestive tract live only a few days and females lay but few eggs or none it has sometimes been possible to obtain offspring from them. These offspring are usually normal.

Strain Showing Hereditary Deficiency in Genitalia and Digestive Tract.

Hereditary deficiency in genitalia and in digestive tract arose from the combination of material from Lancaster, Pennsylvania, and Iowa City, Iowa. No deficient occurred in the direct line of Iowa City ancestry which included 598 males and 543 females running back to the original female. An I male was crossed to an L female, stock 6. An F_2 male from this cross was mated to an L female, stock 10. This resulted in fifty-three normal males and 130 normal females, besides one female with deficient digestive tract. This is the first occurrence of the character in the ancestry. A sister of this female produced 224 normal males one of which was mated to an I female. Fifty normal males and ninety-six normal females resulted. One of these females produced 139 normal males, five deficient males and one impaternate female. (Female from unfertilized egg—from 934.5.)

Descendents of this impaternate female include in all 209 fraternities. Inbreeding was carried on by simply isolating individual females after they had had a chance to mate with their brothers. Ratios of deficients in these fraternities varied erratically and a large number of insects died immature. The latter were recorded without reference to sex.

Ratios of deficients among the males in the 209 fraternities were arranged in a frequency distribution. Grouped to the nearest 2 per cent. they ranged from 0 to 58 per cent. with frequencies 57, 6, 4, 9, 11, 21, 19, 8, 6, 5, 7, 5, 3, 8, 6, 6, 5, 5, 0, 6, 2, 0, 0, 2, 0, 2, 0, 1, 1, 2, besides two fraternities each consisting of a single deficient individual.

131 of these fraternities included females with ratios of deficient ranging from 0 to 34 per cent. Corresponding frequencies were 80, 1, 10, 9, 7, 2, 4, 7, 2, 2, 1, 0, 0, 3, 1, 0, 0, 2. There were also two small fraternities, one with one deficient to one normal and the other with four deficient to two normal.

Percentage of wasps dying immaturely were calculated for the 209 different fraternities. These ranged from 0 to 32 with frequencies 125, 11, 17, 19, 12, 5, 6, 2, 3, 1, 3, 0, 1, 1, 2, 0, 1.

There were 10,335 males and 2,165 females in the entire group.

Frequency According to Age of Mother.

In a special line of this deficient stock consisting of thirty-seven cultures there were 2,094 males with 540 or 25.788 per cent. deficient, 468 females with 43 or 9.188 per cent. deficient, and among the total of 2,732 there were 170 or 6.223 per cent. dying immature.

Females are transferred every four or five days and culture vials labelled *a*, *b*, *c*, etc. Thus if offspring be summarized according to letter of vial average changes in progeny according to age of mothers may be obtained. Table *A* and Chart 1 show

TABLE *A*.

OFFSPRING OF STRAIN DEFICIENT IN GENITALIA AND DIGESTIVE TRACT ARRANGED ACCORDING TO AGE OF MOTHER (VIALS).

	Vial.							
	a	*b*	*c*	*d*	*e*	*f*	*g*	Total.
Males, Total......	694	458	370	318	148	70	36	2,094
Deficient......	109	135	129	120	34	10	3	540
Females, Total.....	168	196	96	8				468
Deficient......	12	17	14	0				43
Total.............	888	706	513	358	160	71	36	2,732
Immature deaths...	26	52	47	32	12	1	0	170

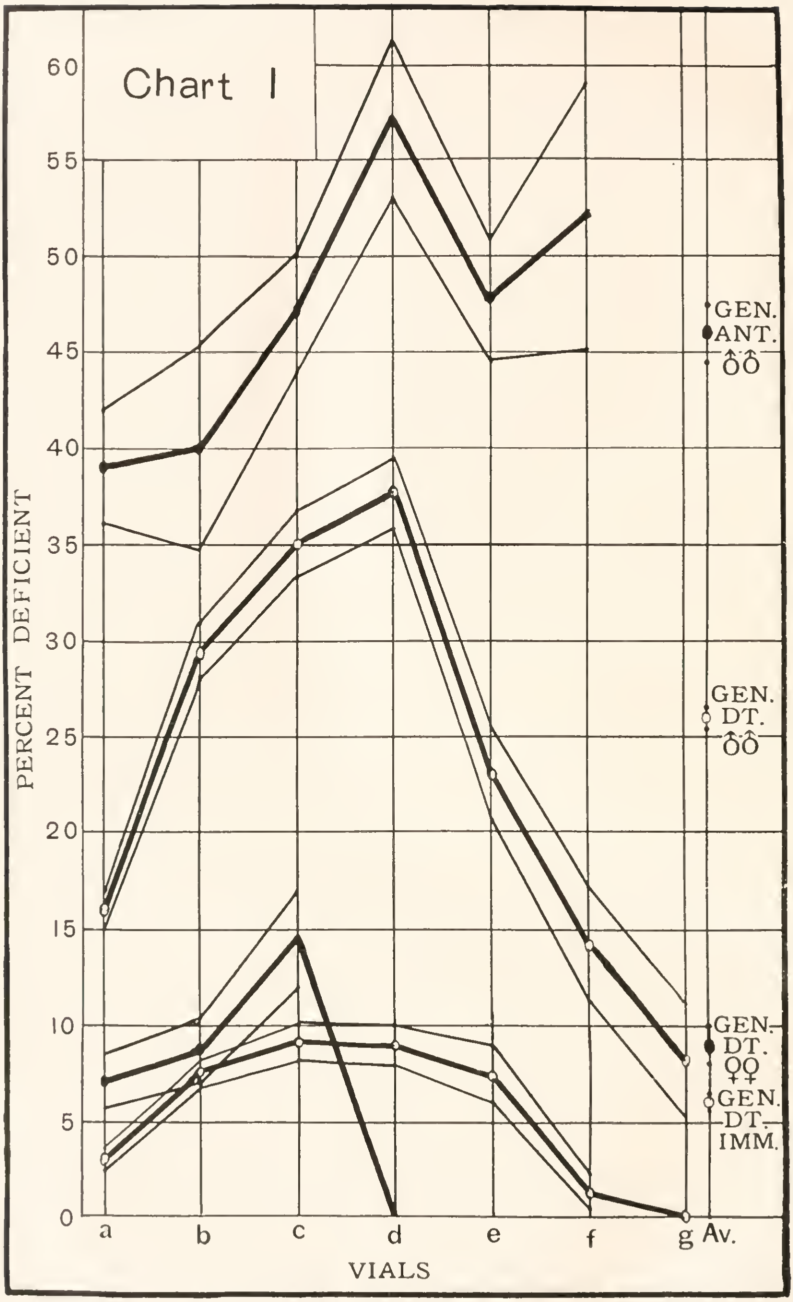

CHART 1. Percentage of deficient offspring according to age of mothers. Culture vials through which mothers were successively transferred are denoted by *a*, *b*, *c*, *d*, *e*, *f*, *g*. Mothers remained in each vial between four and five days. *Gen. Ant.* and *Gen. Dt.* denote offspring from mothers of strains in which deficiency in genitalia was associated with deficiency in antennæ and in digestive tract respectively. For actual numbers see Tables *A* and *D*.

TABLE *B*.

OFFSPRING DEFICIENT IN GENITALIA OR DIGESTIVE TRACT ARRANGED ACCORDING TO TYPE OF DEFICIENCY AND AGE OF MOTHER (VIALS).

	Vial.							
	a	*b*	*c*	*d*	*e*	*f*	*g*	Total.
Males								
(Genitalia reduced): actual	6	10	7	5	1	0	0	29
per cent	5.5	7.4	5.4	4.2	2.9	0	0	5.37
(Genitalia absent): actual	74	100	79	85	22	7	3	370
per cent	67.9	74.1	61.2	70.8	64.7	70.0	100.0	68.52
(Deficient digestive tract): actual	4	0	5	5	1	1	0	16
per cent	3.7	0	3.9	4.2	2.9	10.0	0	2.96
(Deficient genitalia and digestive tract): actual	25	25	38	25	10	2	0	125
per cent	22.9	18.5	29.0	20.8	29.4	20.0	0	23.14
Females								
(Deficient genitalia): actual	2	3	1					6
per cent	16.6	17.6	7.1					13.95
(Deficient digestive tract): actual	8	13	11					32
per cent	66.6	76.4	78.6					74.42
(Deficient genitalia and digestive tract): actual	2	1	2					5
per cent	16.6	5.8	14.3					11.63

results of such summarizing for this group. It may be noted that tendency toward production of deficiency is relatively low when mothers are young but increases up to the tenth to fourteenth day when it rapidly falls. Essentially the same form of curve is seen for deficient males, deficient females, and immature deaths but data for females are rather meager.

The various types of deficients as they occurred in the different bottles are recorded in Table *B*.

By glancing at the percentages in this table it may be seen that there is no consistent and significant change in ratio of any type of deficiency according to the age of the mother. The continuous increase in females with deficient digestive tract is not to regarded as significant because of small numbers.

Crosses of Deficient and Normal Stocks.

All females of the deficient line were lost through their failure to produce daughters. Their sons were crossed with normal L stocks. Daughters were back-crossed to the same males for three generations. Although the males came from the selected material and were used as parents for four successive generations, the ratio of deficient did not exceed two per cent. 6,954 wasps of the third generation were bred. Wasps were deficient in genitalia alone, ninety-six; in digestive tract alone, twenty; in genitalia and digestive tract, fourteen.

A male from the selected deficient line was crossed to a female from Iowa City stock 11. The male had orange eyes, wrinkled wings, defective r_4 vein, and yellow mesosternum. Stock 11 is characterized by black eyes, flat wings, normal r_4 vein and sooty mesosternum. Orange acts as a unit with no overlapping, wrinkled has but little. Defective and sooty are variable multiple factor characters in this cross.

The deficient in F_2 numbered among 2,252 males only thirty-five or 1.55 per cent., and among 957 females only five or 0.52 per cent.

The thirty-five males included nineteen orange, fifteen wrinkled, and twenty sooty. Among the twenty in which venation could be determined ten had defective r_4. There is thus no association between deficiency and the characters mentioned.

These males were classified as genitalia aberrant, one; genitalia lacking, nineteen; digestive tract deficient, nine. Four females were deficient in digestive tract, one in genitalia and digestive tract.

Strain Showing Hereditary Deficiency in Genitalia and Antennæ.

Deficiency in genitalia and in antennæ characterized cultures derived from a female of Lancaster stock 10. Deficiency in digestive tract did not occur among these with the exception of a single male (of fraternity F_1A in Table *C*). This specimen was also deficient in genitalia and antennæ.

Table *C*.

Males Showing Deficiency in Antennæ and Genitalia. Three Generations Descended from "Mutant" Female from Stock 10.

Fraternities.	Total.	Deficient.			Total Deficient.	Per Cent. Deficient.
		Genitalia.	Antennæ.	Genitalia and Antennæ.		
F_1A	104	6	4	21	47*	45.2
F_2A	3				0	0.0
B	9				0	0.0
C	22				0	0.0
D	72		1		1	1.4
E	115		2		2	1.7
F	39		1		1	2.6
G	66	3	2		5	7.6
H	10		1		1	10.0
I	12			4	4	33.3
J	30	2	3	8	13	43.3
K	76		4	30	34	44.7
L	112	8	1	47	56	50.0
F_3A	81				0	0.0
B	88	1	2		3	3.4
C	151		6	62	68	45.0
Total F_1A F_2I–L F_3C	485	16	18	172	222*	45.8
Total F_2A–H F_3A–B	505	4	9		13	2.6

* Sixteen unclassified deficient of vial *a* are included here.

The stock 10 female was isolated as a virgin August 1924. After passing through vials *a*, *b*, and *c* she was mated to her sons from *a*. Daughters appeared in the succeeding bottles. Her progeny totalled 104 males, twenty females, and one immature.

All the females were set. Eight proved sterile and twelve fertile. In later generations female sterility also appeared. In the total line there were seventy-two females set of which twenty-one failed to produce offspring although they lived the average length of time.

There were 473 females in the whole line of which one had deficient genitalia, one had tapering antennæ and one showed both defects.

There were recorded only four immature deaths.

Males of F_1, F_2, and F_3 from one F_2 fraternity are recorded in Table *C*. Other F_3 males and males of later generations consisted of 489 normal and eight deficient. These eight occurred in F_3 after which no more appeared.

The fraternities of males recorded in Table *C* evidently fall into at least two groups.

The six fraternities F_1A, F_2I–L, and F_3C are the only ones showing males with deficiency both in genitalia and in antennæ. They are highly deficient, the ratio averaging 45.8 per cent.

Deficiencies in antennæ and in genitalia are highly correlated in this group for they both occur in 172 cases out of 206. (Vial *a* of the F_1 generation could not be included in this total as the deficient were not separated into the three groups.) The remaining ten fraternities of Table *C*, F_2A–H, F_3A–B, with 505 males have only thirteen or 2.6 per cent. deficient. None of these is deficient in both characters.

Frequency According to Age of Mother.

The males of the six fraternities showing High Deficiency have been summarized with respect to vials in order to test influence of age of mother on ratio of deficient offspring. Table *D* gives results of this summary and Chart 1 shows percentage frequencies.

The rise from *a* to *d* is, as in the case of deficiency in genitalia, associated with deficiency in digestive tract, clearly significant. The analogous drop to *e* is of doubtful significance; the subsequent rise of no significance.

Table *D*.

Males of Strain Deficient in Genitalia and Antennæ Arranged According to Age of Mother (Vials).

	Vial.						
	a	*b*	*c*	*d*	*e*	*f*	Total.
Total	116	40	123	72	111	23	485
Deficient	45	16	58	38	53	12	222

It may be concluded that age of mother is an effective factor in determining this type of deficiency.

Discussion and Summary.

Deficiency or reduction in antennæ, in external genitalia, and in posterior part of the digestive tract occurs occasionally in individuals of normal stocks.

Two cases of marked hereditary deficiency present some interesting points of difference. In one, deficiency in genitalia was associated with deficiency in digestive tract and many immature deaths occurred. The trait was increased and maintained by selection but very few fraternities approximated 50 per cent. even among the males. Great variability in ratios in the different fraternities and failure to recover the stock by breeding normal up to males of selected deficient indicate that the character is genetically complex.

In the other case, deficiency in genitalia was associated with deficient antennæ. There was no increase in immature deaths over those normally occurring but much female sterility. The line originated from a female from stock supposedly normal. Fraternities fell into at least two groups. In one the deficient averaged 2.6 per cent. of the total and were deficient either in genitalia or in antennæ but not in both. In the other they averaged 45.8 per cent. of the total, the majority of them being deficient both in antennæ and in genitalia. The facts indicate genetic complexity.

Percentage of deficient offspring varies according to age of the mother in both cases. It increases up to approximately the tenth or fourteenth day of adult life and then decreases. No consistent change in type of deficiency according to mother's age could be detected.

Possible explanations of this rise and subsequent fall of ratio of deficient during life of mother may be considered.

Cytoplasm or yolk of eggs might differ according to some rhythm in the life of the female. The influence would have to be carried through the larval period and up to the time of the formation of the organs in the pupa.

Something in the food of the larva might vary according to the age of the mother, some ingredient of the fluid injected at time of stinging the caterpillars.

Neither of these theories involves genetic change in maternal contribution.

A third explanation may be suggested. It has been shown that linkage in *Drosophila* varies with age of mother. If this is true in *Habrobracon* a difference in genetic composition of offspring according to the mother's age may be brought about. In order to test this possibility records on a lethal factor linked with orange were reinvestigated (Whiting, P. W., 1921) and the five fraternities involving linkage were summarized according to vials. Results are given in Table *E*. The difference between vials *a* and *b* is of no significance. There is an increase in cross-overs from *b* to *c*, 13.5 ± 4.6 per cent., which is probably significant and a decrease after *c*, 16.1 ± 5.0 per cent. which is also probably significant. If this rise and fall of crossing-over according to age is proved when further linked factors are found, it may be the most reasonable explanation of change in ratio of deficient.

TABLE *E*.

CROSS-OVERS OF A LETHAL LINKED WITH ORANGE ARRANGED ACCORDING TO AGE OF MOTHER.

	Vial.				
	a	*b*	*c*	*d–f*	Total.
Cross-overs/Total	14/73	18/108	19/63	8/57	59/301
Per cent	19.2 ± 3.1	16.7 ± 2.4	30.2 ± 3.9	14.0 ± 3.1	

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EXPLANATION OF PLATE I.

All figures have been drawn with camera lucida. Drawings have been reduced two thirds. Final magnifications are × 32 for Figs. *A–H*, × 15 for Figs. *I–M*.

FIGS. *A*, *B* AND *C*. Ventral view of head, ventral and posterior views of tip of abdomen of deficient male (Freak 60).

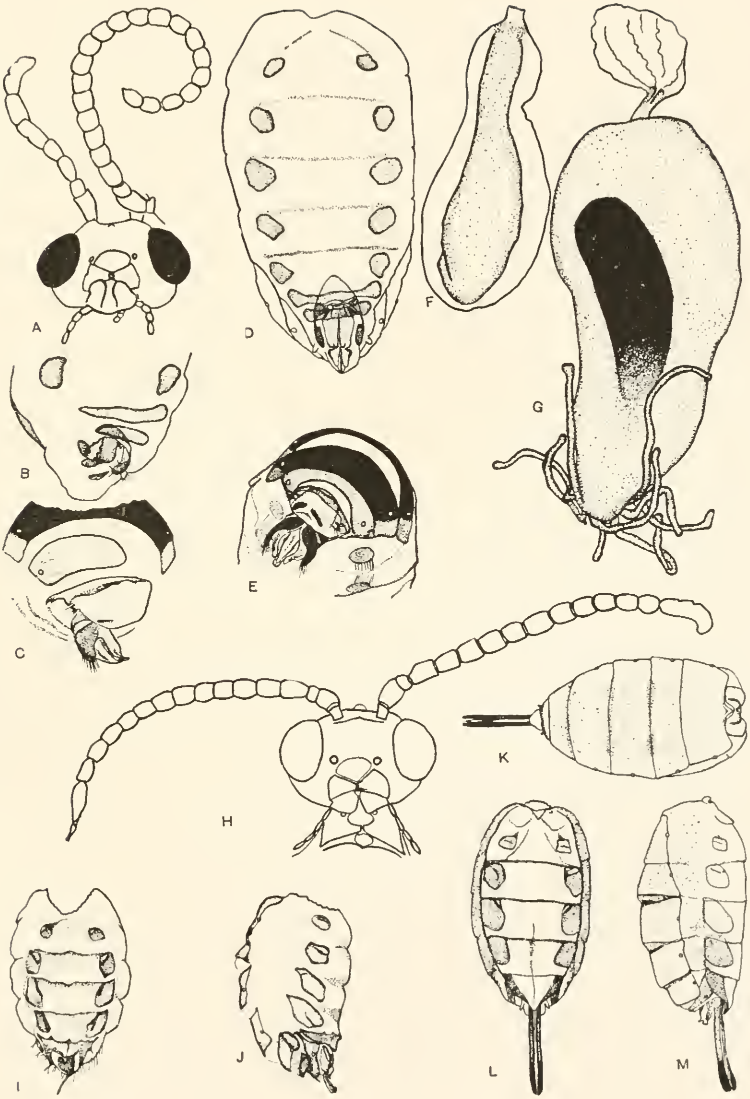
FIGS. *D* AND *E*. Ventral and posterior views of abdomen of normal male.

FIGS. *F* AND *G*. Abdominal portion of digestive tracts of deficient female (Freak 21) and deficient male (Freak 20).

FIG. *H*. Anterior view of head of deficient male (from 1171.3*c*).

FIGS. *I* AND *J*. Ventral and right views of abdomen of deficient female (Freak 6).

FIGS. *K*, *L* AND *M*. Dorsal, ventral and right views of abdomen of normal female.


A
B
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D
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P. W. WHITING.

GAMETIC MEIOSIS IN *MONOCYSTIS.*

GARY N. CALKINS AND RACHEL C. BOWLING.

The monocystids of the earthworm, probably the commonest and most frequently found type of gregarine, have never been completely monographed, although an excellent start in this direction was made by Hesse (1909). Cytological studies have been made by Wolters (1891), Cuénot (1901), Shellack (1912), and Mulsow (1911), the latter finding typical gametic meiosis or chromosome reduction in a species which he regarded as new and to which he gave the name *Monocystis rostrata.* In this form which he named from the presence of an attenuated proboscis-like end of the cell, he found eight chromosomes of characteristic form and size. This number of chromosomes was found in the first nuclear spindle of the gamont after metamorphosis of the vegetative nucleus, and the same number was present in the successive spindles of the succeeding progametic nuclei and until the final division when the gametes are formed. In this ultimate division Mulsow found that whereas eight chromosomes are present in the nuclear plate of the mitotic figure at the metaphase only four chromosomes are present in the daughter plates of the anaphase stage and only four chromosomes are present in each of the two resulting gametes. Here then the haploid number of chromosomes is characteristic of only one set of cells, viz. the gametes, of the entire life cycle. Upon fusion of two gametes the diploid number is restored and this diploid number is characteristic of the entire vegetative cycle including all of the progametic nuclei. The actual reduction in number of chromosomes according to this account agrees in point of time with the corresponding meiotic phase in the great majority of the metazoa, a type which Wilson describes as gametic meiosis.

A very different time of reduction in chromosome number was described by Dobell in connection with gamete formation, fertilization, and metagamic divisions of the coccidian *Aggregata eberthi*, and by Jameson in connection with the gregarine *Diplo-*

cystis schneideri (Dobell and Jameson, 1915). This joint paper was followed later by individual papers on each of the two types (Dobell, 1925; Jameson, 1920). In both of these types the authors found that the progametic divisions are all alike including the last or gamete-forming division, in having nuclei with the same number of chromosomes, and that this number is the same in all of the vegetative stages. The zygotes, alone of all cells of the vegetative cycle, have the double or diploid number of chromosomes. Meiosis or reduction in number of chromosomes takes place with the first mitotic division of the zygote, a type known as zygotic meiosis. The individuals which one ordinarily encounters of these two species are therefore haploid as regards chromosome number.

From these findings Dobell and Jameson make the generalization that zygotic meiosis is probably universal in Gregarinida and Coccidia and they interpret Mulsow's account of reduction in *Monocystis rostrata* as evidence of confusion by him of two species of *Monocystis*, one with eight the other with four chromosomes. It seems a little premature to say the least to sweep away contradictory evidence in this fashion when a re-examination of *Monocystis* in the light of Dobell and Jameson's work would clear up the matter. The cytological results of such a re-examination are presented herewith.

There are many species of *Monocystis* in earthworms some of which are apparently limited to certain species of worms. We have restricted our investigation to the parasites of the seminal vesicles of one species—*Lumbricus terrestris*. Here several species of monocystid gregarines are found, some almost invariably present, others less frequently. We have frequently encountered the large form with a proboscis-like end which undoubtedly corresponds with Mulsow's *Monocystis rostrata* (450 μ long by 220 μ broad). We have not seen the long thread-like form which Hesse names *Nematocystis magna* (*Monocystis magna* Schmidt) and is said to be from 4.5 mm. to 5 mm. long and only 50 μ in diameter. We have not seen *Monocystis striata* Hesse with its longitudinal grooves and striations which are described as frequently prolonged posteriorly to form a tuft of filaments as in the genus *Zygocystis*. We often find *Monocystis agilis* which can be easily identified and we often find another species which might be regarded as one of the varieties of *Monocystis lumbrici* which are not precisely described in the literature.

Cuénot (1901) Brasil (1905) and Hesse (1909) all remark on the difficulty of tracing a pair of pseudo-conjugants of *Monocystis* back to the individual agamonts which form the subjects of the specific descriptions. Where only one type of gregarine is present in an organ the assumption is safe that the cysts found in the organ come from that one type. When, however, there may be as many as five different species present at the same time, as is the case in the seminal vesicles of *Lumbricus terrestris*, then the problem is real. There is indeed a real danger of confusing sporocysts from different species and of getting a false interpretation of the life history of any one. On the other hand, if the species of *Monocystis* were based upon the size and structure of the sporocysts and sporoblasts instead of upon the agamont stages the difficulties of identification attendant upon multiple infection would be materially lessened.

While an ideal condition would be the indisputable association of a given agamont with its definitive sporocyst in these monocystids, for our purpose such an ideal is unnecessary. In all gregarines two individuals upon reaching maturity become associated in pairs (pseudo-conjugation) and a capsule, called the sporocyst capsule or simply sporocyst, is formed about them (Fig. 1). These gamonts then proceed to form gametes by repeated divisions of the originally single nucleus in each, the gametes ultimately appearing as a zone of small nucleated cells budded out from the periphery of the gamonts. The gametes from one parent cell then meet and fuse with the gametes from the associated cell. The zygotes thus formed by fusion two by two are practically equal in number to the total number of gametes formed by either one of the associated parent cells. Each zygote forms its individual capsule termed the sporoblast capsule within which the zygote nucleus, an amphinucleus, divides a certain number of times, usually three. The eight resulting nuclei become the nuclei of the definitive germs termed sporozoites.

It is no more difficult to recognize different specimens of the same type of cysts and sporoblasts than it is to recognize different individuals of the same species of *Monocystis*. The structure of the agamont protoplasm, the nature of the sporocyst capsule, the appearance of the nuclei in size and character together with the number of chromosomes, are factors involved in the recognition of a cyst of a certain species. We have been no more fortunate than our predecessors in tracing the indisputable connection between a specific agamont and the sporozoites which it ultimately forms and for this reason we beg the question of the particular species of *Monocystis* with which we are dealing. We are confident however that we have followed the history of one single species.

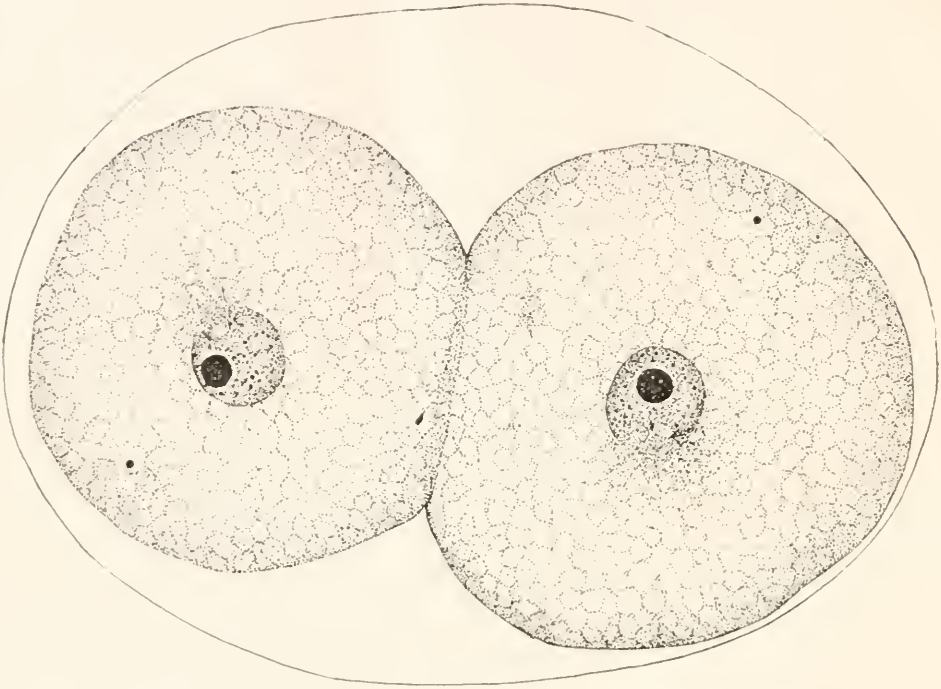

FIG. 1. *Monocystis* species. Two gamonts in pseudo-conjugation in sporocyst capsule. Nuclei beginning metamorphosis. Minute spindles in substance of centrosphere. Smear preparation. Camera lucida. × 900.

We have chosen for particular study the cysts containing the largest sporoblasts and the largest gametes because their nuclei give the clearest pictures of the nuclear contents. The total number of gametes formed by both gamonts in this species is approximately 130 and the number of sporoblasts is approximately 65. The protoplasm is uniformly alveolar, clear, and with alveoli of medium size. The nucleus of the gamont is spherical and contains a single spherical endosome.

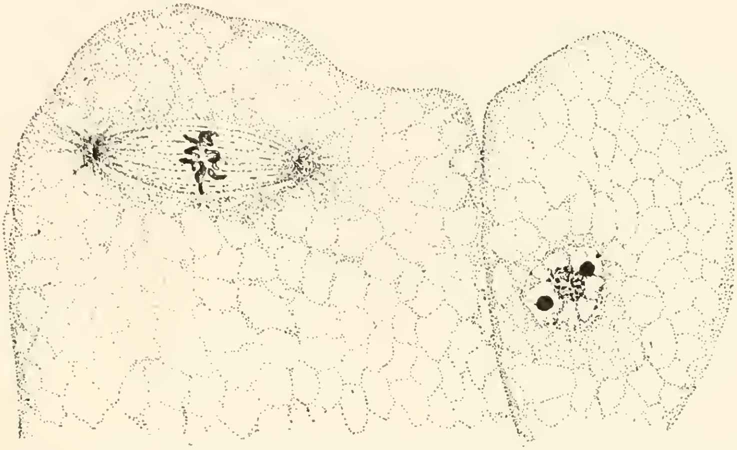

FIG. 2. First pregametic spindle with 10 chromosomes in one gamont; portion of disintegrating nucleus in the other. Section. Camera lucida. × 2650.

The material was fixed in hot Schaudinn's fluid consisting of a saturated solution of bichloride of mercury in absolute alcohol without and with 5 per cent. of glacial acetic acid. Seminal vesicles were removed entire from richly infected earthworms and immersed after puncturing, in the killing fluid where they were left for from two to four hours. They were then treated for twenty-four hours with iodized alcohol, embedded in paraffine and cut in sections from three to four microns thick. The sections were stained with iron hæmatoxylin without or with a counter stain. Other stains were tried but the best results were obtained with iron hæmatoxylin.

The metamorphosis of the gamont nucleus begins with the condensation of the chromatin and formation of the chromosomes. At this stage the latter are feebly staining and even in the metaphase of the first division they do not stain with the same intensity as in the later progamous divisions or as they do in the developing zygote. An astrosphere is condensed from the cytoplasmic reticulum at one pole of the nucleus and the nuclear membrane disappears at this region. We did not observe a stage in which a single centriole could be found in the astrosphere, the earliest stage seen being a minute spindle which appeared to be formed in the substance of the astrosphere in the region which had been occupied by the now dissolved nuclear membrane (Fig. 1). This spindle enlarges until it occupies fully one half of the diameter of the cell (Fig. 2). The short, rod-shape or slightly curved chromosomes are ten in number. The daughter nuclei when fully divided may be separated by the full diameter of the cell, and remains of the spindle substances trail out behind them marking the path of the spindle (Fig. 3).

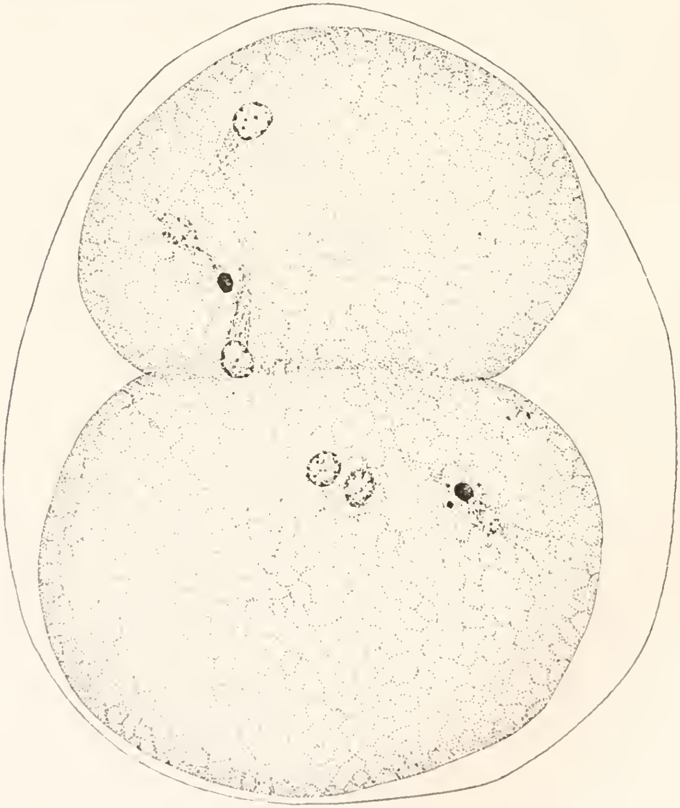

FIG. 3. Two gamonts after first pregametic nuclear division. Smear. Camera lucida. × 900.

The subsequent spindle figures are characterized by the increasingly intense staining capacity of the chromosomes while the size of the nuclei decreases. There is a tendency for the daughter nuclei after each division to approach the periphery and the final progamous nuclear spindle lies in a plane tangential to the surface (Figs. 4 and 5). In some cases the spindle occupies a bud which stands out from the surface in characteristic fashion

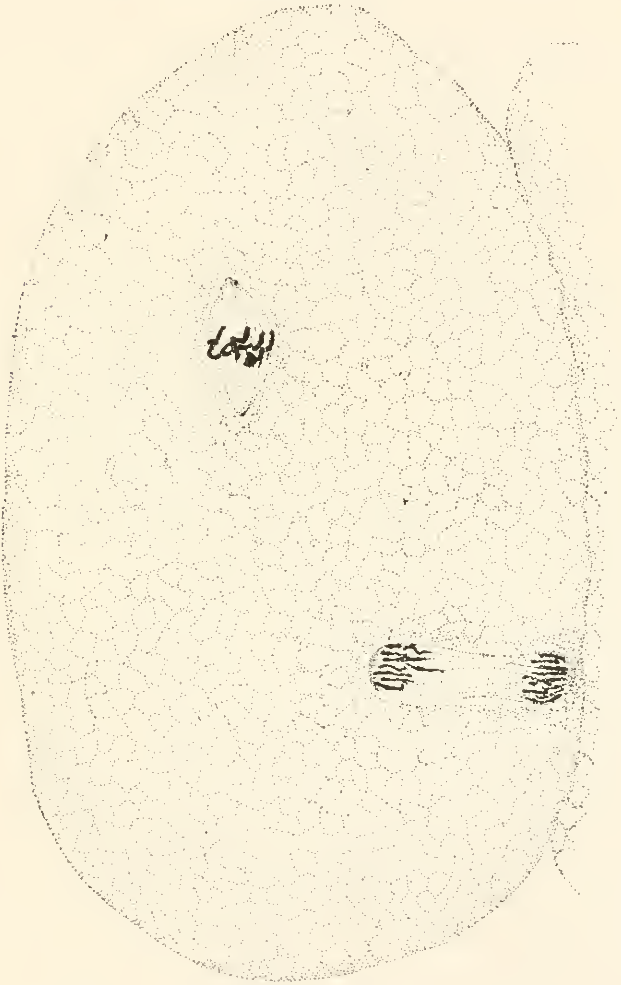

FIG. 4. Third pregametic division of nuclei with 10 chromosomes. Section. Camera lucida. × 1750.

(Fig. 7). Such tangential nuclear figures are typical of the final progamous division, and their products become the gamete nuclei.

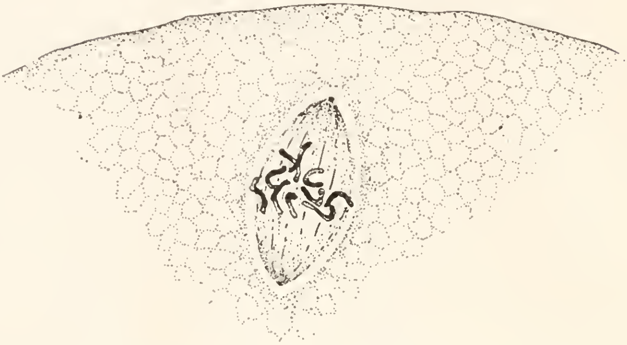

FIG. 5. Detail of a spindle during the third pregametic division. Section. Camera lucida. × 2650.

In the tangential nuclei as in the earlier pregametic nuclei we have seen no evidence of a prophase skein stage. Metaphase stages of division are frequently found (Fig. 6) and early and late

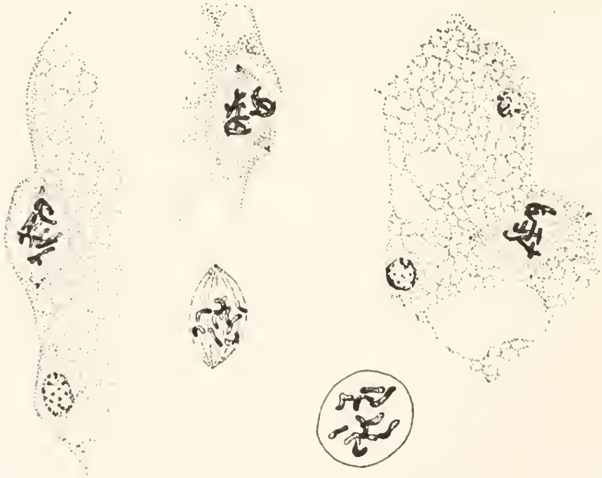

FIG. 6. Group of spindles in the metaphase stage of the last pregametic division, one showing polar view of a nuclear plate. From different individuals; all with 10 chromosomes. Sections. Camera lucida. × 1750.

anaphase stages. In these the number of chromosomes is always ten (Figs. 7 and 8). In the metaphase stage of the tangential spindles there are likewise ten chromosomes, but these ten do not divide longitudinally. They are separated into two groups of five chromosomes each, easily distinguishable in the anaphase groups (Figs. 7 and 8). Reduction in number of chromosomes from 10 to 5 therefore takes place with the last progamous division.

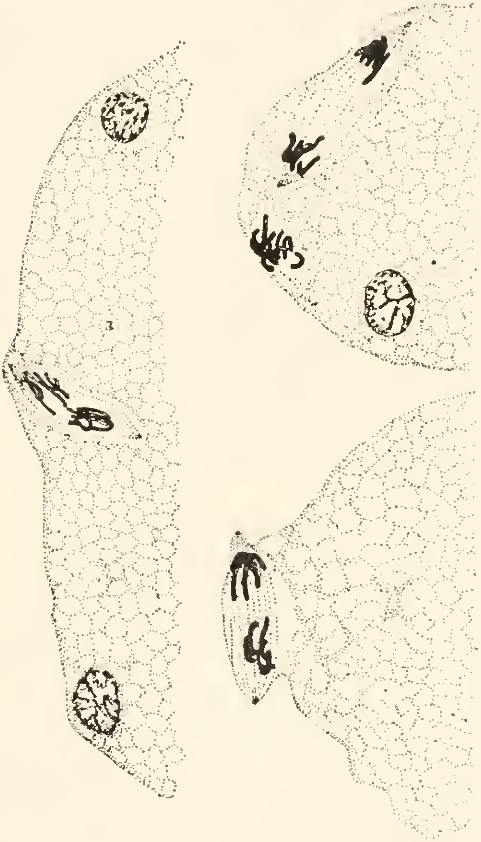

FIG. 7. Group of spindles in the anaphase stage of the last pregametic division; from different individuals. 5 chromosomes in the daughter plates. Sections. Camera lucida. × 1750.

The chromosomes are not sufficiently different in shape and size to furnish any evidence of coupling. Two daughter groups are represented in Fig. 8; some are U-shape others are usually somewhat sigmoid in character, but the differences are not so

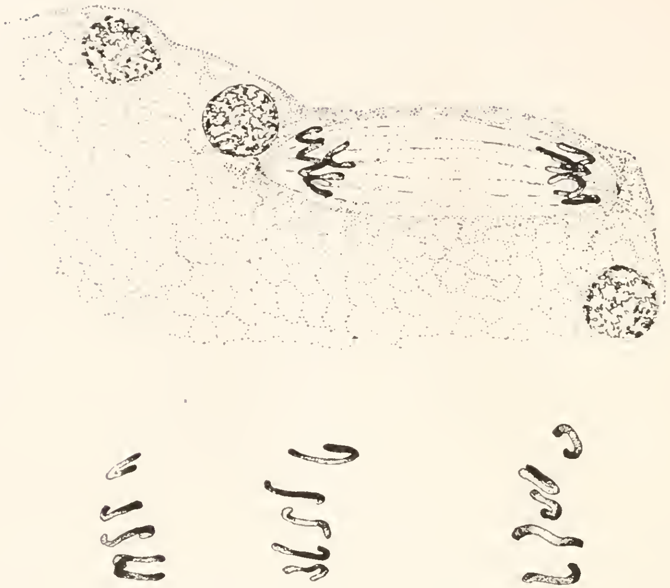

Fig. 8. Anaphase stage of last pregametic division and 3 groups of chromosomes from other spindles in the same stage. Sections. Camera lucida. × 2650.

marked as in the case of *Aggregata* or as in Mulsow's *Monocystis rostrata*.

The gametes are relatively large, spherical to ovoidal in shape and without constant dimorphism although minor variations in

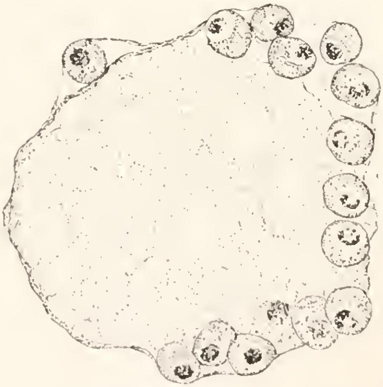

Fig. 9. Characteristic grouping of gametes around periphery of gamont. Section. Camera lucida. × 600.

size may be observed even in gametes from the same gamont (Fig. 9). The nuclei are usually near the anterior end of the cell

(Fig. 10) and when two gametes come together, fusion begins at this nucleated end. The nuclei fuse and the zygote secretes a delicate plastic membrane which is transformed later into the rigid sporoblast capsule (Figs. 11, 12 and 13). At this early period of the zygote the form is quite variable owing to the plasticity of the membrane (Fig. 15). The amphinucleus swells and becomes more conspicuous by reason of the more intensely staining chromatin. The chromomeres become arranged in a loose spireme which appears to be continuous (Fig. 14). The threads are double and break up into 10 chromosomes. A spindle is formed with definite poles and fibrils but the latter are not as sharply defined as in the progamous divisions (Fig. 15). In the anaphase stage 10 chromosomes are present in each daughter plate (Fig. 14).

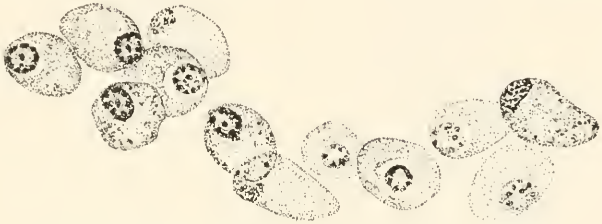

FIG. 10. Group of gametes. Smear. Camera lucida. × 900.

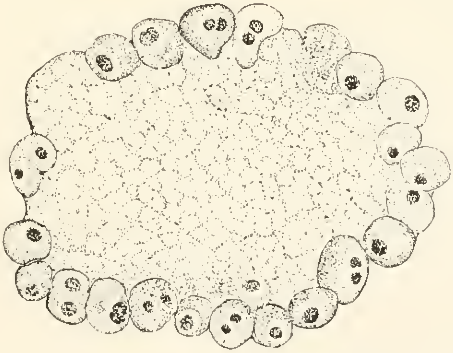

FIG. 11. Characteristic grouping of gametes and zygotes about gametocyte. Section. Camera lucida. × 600.

After this first metagamic division, which is not accompanied by cell division, we were unable to make out any definite spindle

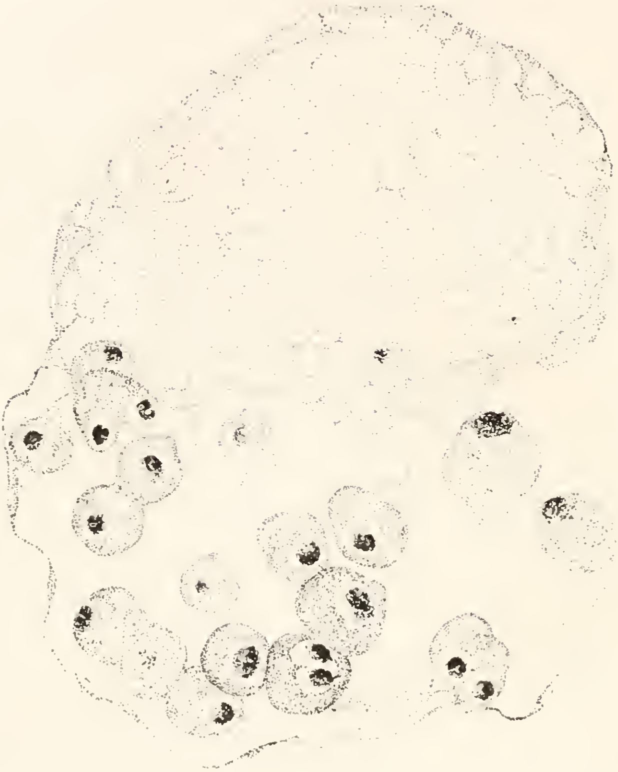

FIG. 12. Group of gametes undergoing fusion to form zygotes. Section. Camera lucida. × 900.

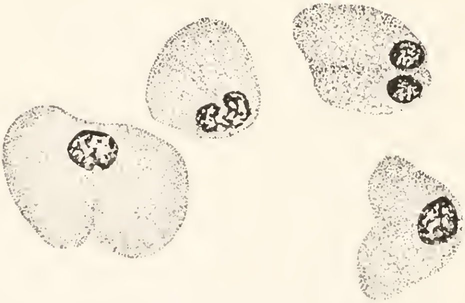

FIG. 13. Group of zygotes with stages in fusion of the nuclei. Section. Camera lucida. × 1750.

formation. The two daughter nuclei soon divide again, an inference formed by reason of the comparatively rare occurrence of the binucleated sporoblast. The two nuclei give rise to four

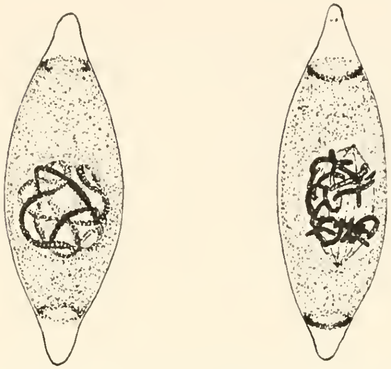

FIG. 14. Young sporoblasts, one with nucleus in spireme stage, the other with nucleus in anaphase stage of division with 10 chromosomes in each daughter plate. Section. Camera lucida. × 2300.

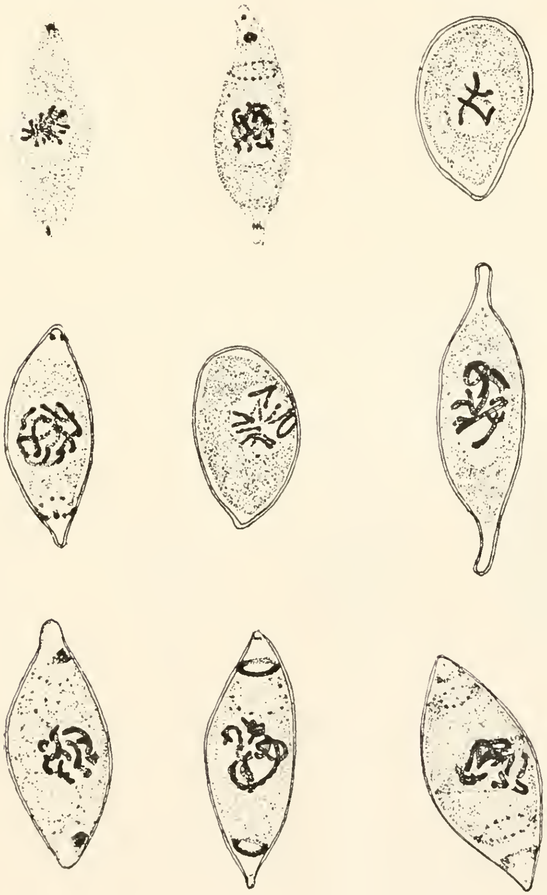

FIG. 15. Group of young sporoblasts in different stages. Sections. Camera lucida. × 1750.

without division of the cell and such sporoblasts with four nuclei are much more common than are the binucleated ones (Fig. 16).

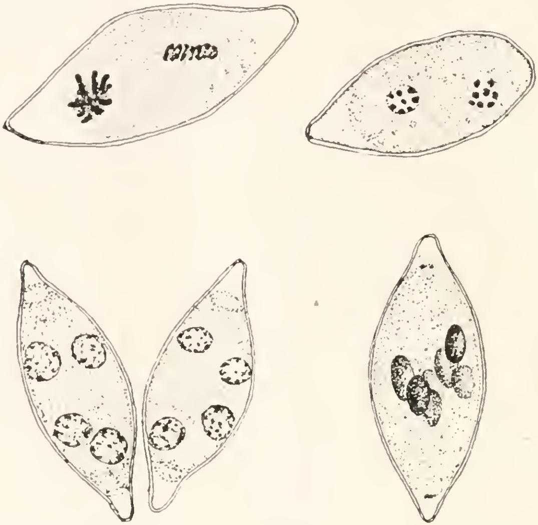

FIG. 16. Group of developing sporoblasts, 2 with 2 nuclei, 2 with 4 nuclei and one with 8 nuclei—no cytoplasmic division. Section. Camera lucida. × 1750.

A third division gives rise to the eight nuclei of the sporozoites and at this stage the cytoplasm divides into eight elongated cells each bearing a nucleus; each is a sporozoite (Figs. 16 and 17).

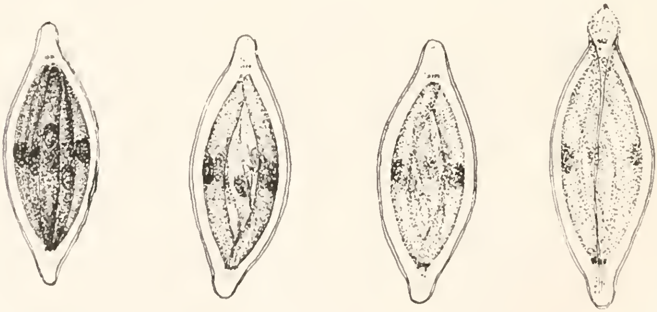

FIG. 17. Group of late sporoblasts from one sporocyst, each with 8 sporozoites In one the sporoblast suture is clearly indicated. Section. Camera lucida. × 1750.

During this later period the sporoblast capsule becomes definitely formed and rigid; near each pole a distinct ring of more intensely staining substance is conspicuous. The origin and significance of these rings will be considered in a later paper. In old sporoblasts a definite suture or groove runs down the sporoblast capsule on both sides from pole to pole, indicating the presence of two valves and a prospective opening by dehiscence (Fig. 17).

The chromosome history of this gregarine thus does not bear out the generalization made by Dobell and Jameson that zygotic meiosis is probably characteristic of all gregarines. It is more probable that both zygotic and gametic meiosis occur in this group, the former probably in forms like *Echinomera hispida* (Shellack, 1907) which has 5 chromosomes, in *Nina gracilis* (Léger and Duboscq, 1909) which has 5 chromosomes, and in *Monocystis ovata* (Shellack, 1912) which has 3 chromosomes, and in other forms with similar odd numbers of chromosomes.

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PRELIMINARY NOTE ON A NEW PROTOZOAN PARASITE OF EARTHWORMS OF THE GENUS *EUTYPHŒUS.*

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In the course of a recent study of the earthworms of Burma a large protozoan parasite of unusual form was frequently encountered. The writer can discover no reference to any parasitic animal of similar form in the literature. The following description will therefore be of interest to zoölogists, although it should be understood that the present paper attempts nothing more than the presentation in a preliminary way of data gathered during a study of the host animal.

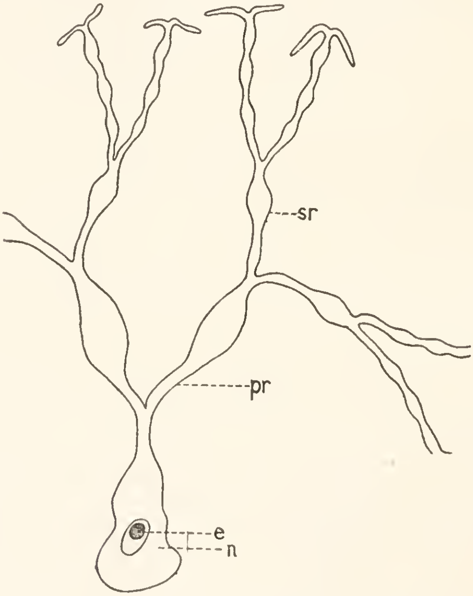

FIG. 1. Part of a trophozoite. (Camera lucida.) *s.r.*, secondary ramus; *p.r.*, primary ramus; *n.*, nuclear membrane; *e.*, endosome.

The parasites may be as long as 4 mm. To the unaided eye the animal presents a smooth creamy-white appearance, or very rarely, a light brownish tinge. The latter color, however, is probably due to a staining action on the parasite of some substance from the host. The large unattached end of the animal is bluntly rounded. At a greater or less distance from this end the body branches into two rami each of which is narrower than the main trunk (Fig. 1). Each of the two primary rami branch again to form two smaller secondary rami. This branching continues until eight or sixteen small ramuli are produced. The region at which ramification begins is not constant in position.

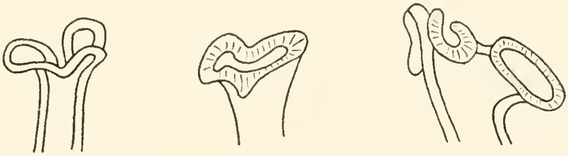

FIG. 2. Sucker-like organs of attachment. (Camera lucida.) × ca. 475.

In one specimen which is 1.4 mm. long the first branching occurs at a distance of about .9 mm. from the blunt end, the second 1.2 mm., and the third 1.32 mm. In other specimens the trunk portion is often much shorter proportionately, rarely even shorter than the primary rami. The ramuli at the attached end are the shortest, the rami usually increasing in length towards the free end of the parasite. The dichotomy is regular in all specimens examined. The ramuli bear groups of irregularly-ovoid, sucker-like objects by means of which the parasite is attached to the host (Fig. 2).

Longitudinal and transverse striations are visible in the ectoplasm with the high powers of the microscope. The transparent fluid endoplasm of the trunk and primary rami is densely packed with ovoid paraglycogen granules. These granules are widely separated in the secondary rami and only very sparsely distributed in the ultimate, transparent branches.

The nucleus (Fig. 3) is a large, ovoid body sometimes visible to the unaided eye in cleared specimens as a minute spot. In large specimens it is .64–.86 mm. in length. The contents of the nucleus are perfectly transparent except for a single, eccentrically located, spherical body of light bluish appearance.

This endosome has either one large, or several smaller, transparent, vacuole-like areas. The nuclei of dead parasites are always found in the unbranched portion.

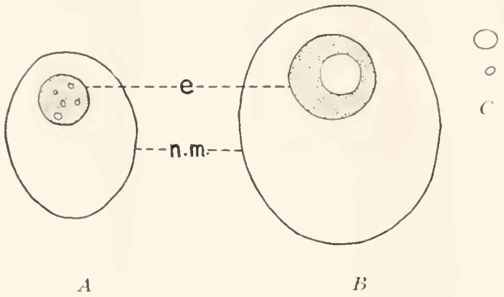

FIG. 3. *A*. Nucleus from trophozoite 1.3 mm. in length. (Camera lucida.) × ca. 335. *e.*, endosome; *n.m.*, nuclear membrane. *B*. Nucleus from larger trophozoite. (Camera lucida.) × ca. 350. *C*. Paraglycogen granules. (Camera lucida.) × ca. 350.

Live attached parasites have not been studied but in living detached forms two series of movements are noticeable. The first series may be described as waves of peristaltic contraction passing along the trunk and rami of the first order. These peristaltic contractions produce a back and forth churning of the endoplasmic contents in the trunk, from the trunk into the primary rami, and back and forth in the rami. By these contractions the nucleus is squeezed to and fro from one end of the trunk to the other. In two of the living specimens examined the nucleus remained in the unbranched portion of the parasite during the whole period of observation. In the other animal the nucleus frequently passed into one or the other of the primary rami. In the primary ramus the nucleus was either rolled back and forth several times or at once passed back into the trunk. The nucleus moved with equal ease and frequency into either one of the primary rami but never passed into the secondary rami which were narrower than the nucleus. It should be noted that in this third animal the ramification began unusually near the blunt end so that the trunk portion was less than one third of the total length of the parasite.

The second series of movements may be called rotation movements and result in a spiral twisting of the primary rami and their branches back and forth on their own axes.

The parasites occur in the cœlomic cavities of one genus of Burmese earthworms, *Eutyphœus* and most commonly in *E. foveatus*. They are found in smaller numbers and more rarely in *E. spinulosus*, *rarus*, and *peguanus*. One hundred per cent. of the *E. foveatus* examined during a period extending over four years have been infected. In the majority of worms of the last named species, large numbers of parasites are found, masses containing 20–40 individuals being not uncommon. The largest groups of parasites occur in segment three, the majority of which are attached to the large nephridial masses of this segment. Other locations are: under the nerve cord in segment four, and on the septa or parietes of segments four to twenty. Those found posterior to segment nine are almost always less than one millimeter in length.

Aikinetocystis singularis, n. gen., n. sp.

Diagnosis.

Uninucleated protozoa parasitic in the cœlomic cavity of certain earthworms of Burma; body cylindrical or columnar with a characteristic, regularly dichotomous branching at the attached end; fixation to the host by means of sucker-like bodies borne on the ultimate branches; nucleus with a single eccentrically placed endosome.

Additional Observations.

Pairs of animals are frequently found attached to each other near the rounded free ends. Among the larger sized forms such pairs are nearly as common as single animals, but do not occur among the smaller parasites. Groups of three to eight animals similarly adherent to each other are also found. Attempts to pull apart the attached animals have always resulted in breaking one or both of the animals near the place of attachment. However no evidence of organic connection between members of a pair or group has been found.

Monocystid-like spores of typical pseudo-navicella form in masses visible to the naked eye are sometimes present in segments three to five. In other specimens in which such masses are not visible similar spores can almost always be found by scraping the body wall of the segments just mentioned. The spores are of two sizes: the larger .02–.023 mm. long, the smaller .007–.008 mm. long. The contents of the spores are coarsely granular. A single transparent vesicular body is present, centrally placed in the small spores, eccentrically placed in the large spores. As no other parasites with a cyst- or spore-forming habit are found in segments three to five and as the spores are practically always present with the trophozoites, it may be inferred, at least tentatively, that they are produced by the trophozoites with which they are always associated.

Numerous specimens of the host have been dissected in every month in which the worm can be obtained but only three cysts from as many worms have been found in proximity to the trophozoites. The cyst in each case was ovoid in shape and about .62 mm. long. A tough membrane enclosed a fluid in which floated two hemispherical bodies in contact with each other on a flattened surface, nearly filling the cavity of the cyst. After rupturing the membrane the gamonts were easily separated from each other. The single hemisphere retained its shape and opacity in clearing fluid. Rupturing the hemisphere by pressure on the cover-glass released granules similar in appearance to those found in the endoplasm of the trophozoite, but no nucleus or spores could be seen. Spore-containing sporocysts have not been found in the anterior segments but cysts about .75 mm. long and .6 mm. wide, densely packed with spores similar in size and appearance to those occurring in the anterior segments are sometimes present in the whitish masses filling the cœlomic cavities of the anal segments.

Worms of the genus *Eutyphœus* occurring in Rangoon suddenly disappear at the end of the rainy season and are not to be found again until after the beginning of the next rainy season. The failure to find the sporocysts in spite of the constant presence of the spores may be due to a coincidence of the sporocyst formation with this winter hibernation period of the host.

SUMMARY.

A new protozoan parasite of the cœlomic cavities of certain tropical earthworms, with a remarkable branched structure of the trophozoite is described. Mention is made of associated spores and sporocysts possibly belonging to the trophozoites.

A CYTOLOGICAL STUDY OF SECRETORY PHENOMENA IN THE SILK GLAND OF *HYPHANTRIA CUNEA*.

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INTRODUCTION.

The general anatomy and histology of the silk glands of lepidopterous larvæ have been carefully studied and described by numerous investigators beginning with the work of Helm ('76), but little purely cytological study of the cells with an aim toward giving an account of the mechanism of secretion was reported before the investigations of Maziarski ('11) and later of Nakahara ('17). Since that time, few papers have appeared based upon studies of the silk gland, with the exception of an account by Yamanouchi ('22) describing a morphological study of silk secretion.

In previous investigations of silk secretion, studies have been made only upon glands of caterpillars which spin a cocoon at a certain period in their life history. Such study is of value in observing glandular function and development during the progressive stages preparatory to and at the time of activity. In order to best study the actual process of secretion, the optimum material would naturally be found in a form which secretes actively over a comparatively long period of time.

The fall webworm, *Hyphantria cunea*, is ideally adapted for the study of active secretion, for the caterpillars live together in colonies, secreting the web continually throughout larval life in the process of securing food. This form, therefore, was selected for the present investigation and by the application of cytological methods, an attempt has been made to determine the cellular organization which is concerned with secretory phenomena in the silk gland.

Work upon this problem was for the most part carried on during 1925 and 1926, at Washington University, St. Louis, under the direction and supervision of Prof. Caswell Grave. It is with great pleasure that I take this opportunity to express to Dr. Grave my sincere appreciation for his advice, criticisms, and valuable suggestions.

MATERIAL.

The spinning-glands of the larval *Hyphantria cunea*, like those of all lepidopteran larvæ, consist of two long tubes, easily distinguishable by their glassy appearance, situated on either side of the nerve cord and below the digestive tract. Each tube is a cylindrical, folded body, averaging between 6 and 8 mm. in length, or slightly less than the entire length of the caterpillar which averages about 1 cm. In total mounts of a spinning-gland, three arbitrary divisions may be distinguished, characterized by differences in appearance, and, in section, by structure (Text-Fig. *D*); the posterior, or secreting portion lying between the eighth and eleventh segments and terminating blindly; the middle portion or reservoir, bending posteriorly to the ninth or tenth segment, then folding anteriorly to the fifth segment; the anterior, or conducting portion extending straight forward to the head where the two tubes converge and unite to open at the apex of a median cylindrical organ, the spinneret.

Each tube consists of a single layer of epithelial cells (Imms, '25, p. 407), varying in shape and size according to position in the tubule. The cells are arranged in two longitudinal rows around a central lumen. The nucleus of each cell is characteristically branched and very irregular. Externally, the gland is covered by a thin membrane, the tunica propria, and internally, the lumen is lined by the thicker tunica intima.

The cells of the posterior region are relatively large and irregular in form and contain a greatly branched nucleus. The inner cell wall is irregular in surface contour, due to numerous drops of secretion in passage between the cell and lumen. The tunica intima is not readily distinguishable (Text-Fig. *A*, *B*). The cells composing the middle region are greatly flattened with a consequent elongation of the nucleus, and possess a clear hyalin, inner border. The cavity of the lumen has increased in diameter and is filled by secreted material (Text-Fig. *C*, *D*).

The cells of the anterior conducting portion are reduced in size, being compressed but less elongated than cells of the reservoir. The nuclei tend to be rounder and less branched, and the intima can be definitely distinguished (Text-Fig. *E*, *F*).

The tracheæ that supply the posterior and middle regions penetrate through the tunica propria into the substance of the cell. It is significant that no intracellular tracheæ are present in the anterior conducting portion of the silk glands.

Color Reactions of Silk Gland Cells with Various Stains and Fixatives.

Fixative and Stain.	Chromatin.	Nuclear Bodies.	Nucleoloid Bodies.	Mitochondria.
Benda:				
Delafield's Hæm., Acid Fuchsin	Blue	Black	Black	
Ehrlich's Hæm., Acid Fuchsin	Blue	Blue	Blue	
Ehrlich-Biondi	Pink	Pink	Orange	
Iron Hæm., Erythrosin	Black	Black	Black	Black
Bouin:				
Delafield's Hæm., Acid Fuchsin	Blue	Blue	Blue	
Ehrlich's Hæm., Acid Fuchsin	Blue	Blue	Black	
Iron Hæm., Acid Fuchsin	Pink	Black	Black	
Champy:				
Altmann, Acid Fuchsin, Methyl gr.	Green	Red	Red	Red
Delafield's Hæm., Acid Fuchsin	Blue	Black	Black	
Ehrlich's Hæm., Acid Fuchsin	Blue	Blue	Blue	
Ehrlich-Biondi	Pink	Red	Orange	
Iron Hæm., Acid Fuchsin	Black	Black	Black	Black
Gatenby:				
Delafield's Hæm., Acid Fuchsin	Black	Black	Black	
Iron Hæm., Acid Fuchsin	Black	Black	Black	Black
Mann-Kopsch:				
Unstained	Yellow	Yellow	Yellow	
Altmann, Acid Fuchsin, Methyl gr.	Reddish	Reddish	Brown	
Regaud:				
Altmann, Acid Fuchsin, Methyl gr.	Green	Red	Green	Red
Iron Hæm., Erythrosin	Pink	Black		Black

Larval *H. cunea* furnished the entire supply of material with the exception of a few glands from *Apatella americana* and *Diacrisia virginica* which were preserved in Bouin and DaFano preparations for comparison. The age of the webworms could not be determined as they had been actively secreting for some time previous to collection as evidenced by the size of the webs. An abundant supply of material was secured from bayberry

Color Reactions of Silk Gland Cells with Various Stains and Fixatives.

Fixative and Stain.	Cytopl. Bodies.	Secretion.		Intima.	Mucoid Sub.	Cytopl.
		Outer.	Inner.			
Benda:						
Delafield's Hæm., Acid Fuchsin	Pink	Light brown	Olive	Pink	Pink	Pink
Ehrlich's Hæm., Acid Fuchsin			Olive	Olive		Pink
Ehrlich-Biondi	Pink	Pink	Blue-gray			Green
Iron Hæm., Erythrosin	Black		Black	Pink	Pink	Pink
Bouin:						
Delafield's Hæm., Acid Fuchsin	Pink	Olive	Pink			Pink
Ehrlich's Hæm., Acid Fuchsin	Pink	Pink	Dark pink	Pink	Pink	Pink
Iron Hæm., Acid Fuchsin	Black	Black	Pink	Black	Pink	Pink
Champy:						
Altmann, A. Fuch., Methyl green	Red	Dark red	Light red	Green	Green	Green
Delafield's Hæm., Acid Fuchsin		Pink	Brown	Pink	Pink	Pink
Ehrlich's Hæm., Acid Fuchsin	Bluish	Pink	Olive	Pink	Pink	Pink
Ehrlich-Biondi	Pink-green	Red-orange	Green-gray	Green	Pink	Green
Iron Hæm., Acid Fuchsin	Black		Black	Pink	Pink	Pink
Gatenby:						
Delafield's Hæm., Acid Fuchsin		Dark brown	Light brown	Pink	Pink	Pink
Iron Hæm., Acid Fuchsin	Black		Black	Pink	Pink	Pink
Mann-Kopsch:						
Unstained	Black		Olive	Brown	Yellow	Yellow
Altmann, A. Fuch., Methyl green	Olive	Olive	Red	Green		Green
Regaud:						
Altmann, A. Fuch., Methyl green	Red	Green	Red-green	Red		Green
Iron Hæm., Erythrosin	Black	Black	Black	Black		Pink

bushes on Nonamessett Island, Mass., between August 13 and 16, and brought into the laboratory at Woods Hole where the animals were kept supplied with food and moisture until killed.

METHODS.

The caterpillars were killed by piercing the head with a pin after which they were immediately fastened in a paraffined dish containing Locke's solution, opened by a dorsal incision, and the glands removed directly with glass needles and placed in fixing fluids. Several fixing solutions were used, namely; Bouin's, Zenker's, Gilson's, for general structure, Benda's, Gatenby's modified "Flemmings" for cell inclusions, Champy's method for preserving and differentiating Golgi bodies and mitochondria, and Regaud for mitochondria.

Transverse and longitudinal sections, 4 micra in thickness, were made of the glands and stained by various methods. The results of different combinations of fixatives and stains may be seen from the chart.

OBSERVATIONS.

1. *The Nucleus.*

(*a*) *Historical.*—The nucleus of larval spinning-gland cells has been found to be characteristically large and greatly branched; the branches extending toward all sides of the cell. This peculiarity of shape early attracted investigators and led them to study the nucleus and its contents in an effort to find some possible relation between its unusual form and the active secretion of silk; but it was not until Korschelt in 1896 published the results of his observations upon living and fixed material that a detailed account of the nuclear content of spinning-gland cells appeared in the literature. From the results obtained through use of a modification of the Ehrlich-Biondi stain, Korschelt concluded that the larger bodies or macrosomes found in the nucleus correspond to the chromatin, and the smaller bodies, or microsomes to nucleoli. Meves ('97) used other staining reagents and came to opposite conclusions. He identified the macrosomes with the nucleoli and the microsomes with chromatin. More recent investigations have seemed to support the conclusions of Meves (Marshall and Vorhies, '06; Vorhies, '08; Maziarski, '11; Nakahara, '17).

(*b*) *Observations*.—Within the nuclear membrane, which can usually be distinguished with little difficulty in the spinning-gland cells of *H. cunea*, bodies of two sizes are always present (except in the anterior conducting region) and with great regularity and frequency a larger, third body is also found (Fig. 5). The smallest bodies are by far the most abundant and tend to fill the entire nucleus. With certain fixatives, Bouin, Zenker, a reticulum can be distinguished, upon which are arranged these smallest bodies. This is not the case, however, after fixation in Regaud, Champy, Benda, or Gatenby, for in the middle and posterior regions of the gland the bodies always appear to be free in the nucleus, forming a compact mass of small granules, and take a basic stain. (See chart.) These bodies have been considered as chromatin. In the anterior region of the gland, these bodies are consistently present although considerably more scattered.

The second group of bodies present in the nucleus exhibits considerable variability both of size and distribution. These are the macrosomes or nucleoli of previous workers, but in this paper, they will be referred to as "nuclear bodies." It is only rarely that these nuclear bodies are found in the nuclei of the conducting cells, and when they chance to be present, are considerably smaller than similar bodies in the posterior or middle regions. No general rule can be given for the shape or dimensions of these nuclear bodies as they tend to vary greatly. Their usual shape is spherical or ellipsoid, and this is typical of their form in the middle region, but toward the posterior end of the gland where there are evidences of active secretion indicated by droplets in the lumen and on the edge of the lumen, these bodies are often elongated and irregular in shape. In size, there is variation in length between 0.5 micron and 2 micra and occasionally a few are larger. Even within the same nucleus, such variation in size is present. The bodies have no special arrangement in the nucleus but tend to be dispersed throughout the area, very often approaching the nuclear membrane. They have even been seen penetrating the nuclear wall into the cytoplasm. Critical observation with high magnification definitely showed nuclear bodies partly within the nucleus and partly in the cytoplasm (Fig. 4). The passage of nuclear bodies into the cytoplasm is a fact that has been described with great care by Maziarski ('11), Nakahara ('17), and earlier workers, as of regular occurrence in the spinning-gland cell; by Saguchi ('20) as occurring in nuclear function of the pancreas cell; and by Ludford ('25) in tissue cultures of fibroblasts of the rats' kidney.

The staining reaction of these nuclear bodies is of interest because of the fact that although it is more or less variable, yet it tends to show greater affinity for acid stains. (See Chart.) It has been upon reactions to stains that all the conclusions in regard to the relation between nucleus and secretion have been made, since it has been regularly found that the nuclear bodies (nucleoli) and apparently related bodies in the cytoplasm exhibit the same staining reactions.

The third class of nuclear bodies found in *H. cunea* has not been described in the literature so far as it has been possible to determine. Because of the similarity between these bodies and the true nucleoli, they will be referred to throughout this paper as "nucleoloid bodies." Meves ('97) mentions the fact that nucleoli may contain several small vacuoles, but in the figures of nucleoli in which he shows this structure, they are no larger than numerous other bodies in the nucleus. Korschelt ('97) in replying to the criticism of Meves, attempts to explain the presence of vacuoles by the hypothesis that the nucleoli or microsomes are derived from the chromatic substance by some transformation evidenced, no doubt, by these vacuoles. Marshall and Vorhies ('06) definitely state that no plasmosome or special structure is formed in the nucleus during secretion in spinning-gland cells of *Platyphylax*, but mention the fact that sometimes the nucleoli may contain vacuoles but no further significance is attached to the observation; nor did Nakahara ('17) consider as important the presence of vacuoles in nucleoli. In the nuclei of *H. cunea*, vacuolated bodies are found to be sufficiently definite to attract especial attention. After carefully measuring a large number of such bodies in all parts of the silk gland, it was found that the average diameter is about 4 micra regardless of position in the gland. Several measured 3 micra in diameter and a very few in the more actively secreting region are as large as 5 micra or 6 micra, while in the anterior region, these bodies average about 2 micra in diameter. This size relation is constant regardless of the fixation used.

Each nucleoloid body contains two or more vacuoles or clear spaces which either do not stain or, if so, only faintly. In a section of a nucleus, usually only one of these bodies is present, rarely two (Figs. 3, 5, 6), and the frequency with which they occur in serial transverse sections 4 micra in thickness leads to the conclusion that not more than two are usually present in a nucleus. This point has been very difficult to determine accurately on account of the uncertainty in deciding where one nucleus ends and the next begins. From longitudinal sections, the evidence was more definitely in support of this conclusion. These nucleoloid bodies are either centrally or just eccentrically placed, and are never found in close approximation to the nuclear wall. Their usual shape is spherical, thus distinguishing nucleoloid bodies from nuclear bodies which tend to vary in shape. From careful study, it has appeared that the presence of nucleoloid bodies is usually associated with an increase in the number of nuclear bodies (macrosomes) either in the same section or the next in series. In the immediate vicinity of the nucleoloid body, the nuclear bodies are smaller, increasing in size toward the periphery of the nucleus (Figs. 3, 5, 6). The periphery of the nucleoloid bodies is not always regular, for often protrusions apparently in the process of pinching off may be seen (Fig. 5).

That the vacuolated nucleolus may give rise to nuclear bodies is not without precedent in observations. Saguchi ('20) in the pancreas cell, describes a very similar process which gives rise to nucleolar corpuscles: p. 355: "As regards the genesis of the nucleolar corpuscles, I am fully convinced that they are derived from the main or side nucleoli." Montgomery ('99) concludes that the nucleolus may act as a nuclear organ either of excretion or storage and that when it has enlarged to a certain extent, it constricts pieces from itself.

The nucleoloid bodies usually react more specifically to basic stains (see chart), but occasionally with Altmann's anilin acid fuchsin, methyl green after fixation by the Regaud method, bodies can be found staining deeply green at the periphery and pink in the center.

(*c*) *Critical.*—From observations upon silk gland cells of *H. cunea*, it would seem that the bodies which migrate from the nucleus into the cytoplasm are not nucleoli, but are rather products of nuclear activity. That this is placing too great a burden upon a single small organ of the nucleus may be argued, but it need not necessarily follow that all the products of secretion are derived from the nucleoloid bodies, but rather that the latter contribute bodies which enlarge in the nucleus, pass out into the cytoplasm where they undergo further modification and later become discharged into the lumen. In order to verify the concluding part of this statement, a general survey of the cytoplasm and its contents is necessary.

2. *The Cytoplasm.*

(*a*) *Cytoplasm.*—The cytoplasmic structure of the silk gland cell as of any cell, is difficult to describe in concrete terms for it is more or less affected by fixation and the exact or true nature is hard to determine. After fixation with the Champy, Gatenby, Benda, or Regaud methods, the cytoplasm is homogeneous and granular, but after Bouin and Mann-Kopsch fixation, a definitely striated appearance may be distinguished, the striæ extending from the lumen toward the periphery. No evidence of fibrils have been found in any case. Yamanouchi ('22) definitely describes fibrillæ in the protoplasm between which are situated granules, especially toward the lumen. These, he explains, are cross sections of fibrillæ and that the entire structure is composed of minute fibrillæ arranged in rows. The usual staining reaction is acidophilic. (See chart.)

(*b*) *Granules.*—In the posterior region of the gland, throughout the cytoplasm, there appear spherical bodies either enclosed in vacuoles or apparently free in the protoplasm (Fig. 1). Maziarski ('11), Nakahara ('17), and others, noted this condition and from the fact that the bodies are often found in close proximity to the nucleus and exhibit the same staining reactions as the nuclear bodies drew the conclusion that the cytoplasmic granules are derived from the nuclear bodies (nucleoli) by migration of the latter. Tanaka ('11) believes the fibroin, or silk core, to be a product of the gland cell substance, visible only when the secretion has accumulated to give rise to fine fibroin droplets which unite to form larger droplets. Yamanouchi ('22) likewise believes that secretion is produced in the cell.

Whether or not these cytoplasmic bodies are produced in the cell or in the nucleus, it seems to be generally concluded that they are directly connected with the products of secretion.

From observation of cytoplasmic granules in the cells of *H. cunea*, it was found that they average between 1 micron and 2 micra in diameter, or approximate the size of the nuclear bodies. The cytoplasmic granules tend to increase in size as they approach the lumen and finally, the droplets of secretion when passed into the cavity coalesce and become of considerable size (Fig. 16). Evidence of active secretion is correlated with an abundance of cytoplasmic bodies present in the cell, but not, as might be expected, with a decrease in the number of nuclear bodies present. It seems, therefore, that if the nucleus does play an active part in the formation of secretion that there is always an excess of nuclear bodies stored in the branched nucleus, and while the drain upon one part may be great, the reserve from another part of the nucleus may supply the demand.

The cytoplasmic bodies are found in all parts of the cell, but usually most abundantly between portions of a nucleus or between the nucleus and the lumen (Fig. 1). These bodies usually react to acid stains, but are generally stained black with iron hæmatoxylin. (See chart.)

That no cytoplasmic bodies are present in the middle section is difficult to determine with absolute certainty. If the nucleus with its nucleoloid bodies and nuclear bodies is responsible for secretion, surely secretion should occur in this region, for both types of bodies are present in the nuclei. Material fixed by the Gatenby method and stained with iron hæmatoxylin and acid fuchsin furnished the only indication that cytoplasmic bodies may be present in the reservoir region. The bodies took a light pink stain very similar to that of the clear, structureless substance surrounding the silk core. These bodies were so indistinct that they may have been the result of poor technique.

Yamanouchi ('22) finds evidence that sericin, or the outer layer of the silk core, is secreted in the posterior portion of the middle region. Blanc ('89) considers that mucous is secreted in the anterior portion of the reservoir in the form of numerous granules, but this point has received little support in subsequent literature.

(*c*) *Mitochondria*.—Throughout the whole of the literature dealing with the silk glands of various lepidopteran larvæ, no reference has been found which even suggests that the workers suspected the presence of mitochondria in the cells. This may be due to the fact that no investigator has employed specific mitochondrial fixatives.

Maziarski ('11) describes certain bodies that are found occasionally in the protoplasm as having the form of short "bâtonnets," and staining deeply. These have a parallel arrangement along the shorter axis of the cell and unite with one another by fine protoplasmic fibrils. The substance of these bâtonnets is refractile and stains with acid stains. Gilson noticed these bodies and thought they were related to functional processes of the protoplasm in that they have the appearance of little tubules filled with secretory substance. Maziarski did not find these bodies consistently present in all of his material.

After fixation with Regaud, and staining with Altmann's anilin acid fuchsin, methyl green, clear-cut evidence of the existence of mitochondria in the cells of *H. cunea* has been obtained.

1. *Posterior Region*.—The mitochondria in the posterior region are very numerous, especially between portions of a nucleus and toward the lumen. In whatever part of the cell the mitochondria may appear, they always show a characteristic orientation with their longitudinal axis toward the lumen. There is no evidence that these bodies have any direct relation to the nucleus for they do not radiate from it or tend to cluster around it (Figs. 1, 2).

Their usual shape is filamentous, rod-shaped or granular. Where active secretion is taking place, long filaments are present, averaging between 3 and 5 micra in length, and about 0.5 micron in diameter. In the more anterior portions of the gland, the mitochondria are found to be shorter, but equally numerous. Mitochondria do not appear to have any direct relation to the cytoplasmic bodies, but the latter are found situated among the mitochondrial filaments.

Material stained with iron hæmatoxylin after fixation in either the Champy or Gatenby method exhibits the presence of structures similar in size, shape and distribution to those observed with Regaud fixation (Figs. 5, 6).

2. *Middle Region.*—In the middle region, mitochondria are always present but their shape is somewhat different from that of the mitochondria in the posterior region. A gradual transition may be seen from the long filaments in the secreting region to the shorter rods in the anterior portion of this region (Fig. 7) and finally, in the true reservoir, the mitochondria become shortened to granules or spherules. The latter have the properties of mitochondria but are somewhat larger in diameter than the mitochondrial granules. It is not probable that the larger spherules represent secretion since in appearance, they more nearly resemble mitochondria than secretion bodies.

The distribution of mitochondria is fairly disperse throughout all parts of the cell, but with a tendency to be more abundant toward the lumen. No definite orientation can be distinguished in the longer rods (Fig. 12).

3. *Anterior Region.*—Bodies taking a stain characteristic of mitochondria are present in the anterior region, but these are always granular and arranged with little definite order (Figs. 8, 9).

4. *Critical.*—From the preceding observations, it is seen that mitochondria are present in the cytoplasm throughout the entire extent of the tubule. Their shape and to a less extent their number varies, however, in the three arbitrary divisions of the gland. Cowdry ('24, p. 318) suggests that mitochondria may serve to some extent as indicators of secretory polarity. In the case of silk secretion, it seems that this is truly the conclusion to be drawn. Secretion can be traced from the cell into the lumen, and mitochondria appear to be streaming toward the

lumen in a very definite way. There is no evidence that mitochondria actively produce secretion but only the indication that they may participate in the process. That mitochondria are present in the reservoir and conducting regions but that here they do not show polarity seems to indicate that mitochondria may be present to perform other functions than that of participating in secretory activity. Activity is occurring in both the middle and anterior regions, although not secretory activity. The middle portion appears to be a storage place for secretion where possible modifications of the secreted material may take place, for example, a condensation and compression. The secreted material then passes through the anterior conducting portion. Tanaka ('11) is the only investigator who has suggested any possibility of the existence of secretory function in the anterior region, and this probably only in the embryonic stages. The usual interpretation is that this region serves as a duct for the passage of secretion. In this case, the presence of mitochondria would seem to indicate that some activity exists within the cell, possibly of general metabolic nature.

(*d*) *Golgi Bodies.*—In preparations of glands fixed by the Mann-Kopsch, and by the Champy method, no structures have been found which could be interpreted as true Golgi bodies. In silk gland cells of *H. cunea* impregnated with osmic acid, numerous fine dark granules appear (Fig. 11), that are distributed very abundantly along the periphery of the cell-body and scattered more generally toward the lumen. These fine granules are present in all regions of the gland and always characteristically more abundant toward the periphery of the cell. In the extreme posterior tip of the gland, the granules appear grouped as a dense border which gradually becomes more disperse toward the lumen. In cells of the secreting region, groups of large, black bodies, spherical in shape and usually situated toward or near the lumen or the periphery are common and may be followed through several sections of a series.

Miss Murray ('26) describes a condition in the follicle cells of the cricket which seems quite similar and her figures compare closely with the condition found in the spinning-gland cell. With regard to her observations, she states (p. 223) that "In the proximal region of some of these cells (follicle cells) there has been seen a nebulous structure composed of very finely divided black particles, which might be considered to indicate an excessive deposition of fat in the tissue. This fine emulsion might conceivably be coarsened at the boundary of the cell and coalesce into fatty globules."

In silk gland cells of *A. americana* and *D. virginica* which were fixed with DaFano's modification of the Cajal method, the same condition was found, but in these cases, there is a greater tendency toward an arrangement of the granules in rows, thus presenting the appearance of striations extending from the lumen toward the periphery, and likewise a greater grouping of granules around the edge of the cell toward the lumen. These bodies tend to concentrate along all sections of tracheæ, thus outlining the course of tracheids very definitely. In these two species, it must be remembered, active secretion of silk only visibly appears at the time of forming the cocoon.

It seems possible that these fine spherical granules may represent by products of cellular activity that are being eliminated. Their condensation along the tracheids and the proximal border of the cell may indicate that such may be the case. In the two species in which the process of secretion is only in the preparatory stage, an accumulation of waste products may be imagined to be occurring in the cell.

3. *The Membranes.*

(*a*) *Historical.*—Before the work of Helm ('76), the existence of a tunica propria, or external membrane, and a tunica intima, or internal membrane was known and had been described, but little was known concerning their structure or function. The tunica propria was quickly dismissed by Helm and those following him as a structureless membrane fitting very closely to the basal membrane throughout the entire extent of the gland that resists to a considerable degree the penetration of fixing fluids. But the tunica intima could not be so easily defined. Helm describes its structure in the anterior region as containing fine radial tubes which he refers to as "Porencanale." The entire intima, according to his observations, represents a cuticular layer which is a modified secretion of the outer body wall and is, in consequence, shed at each moult. The fine canaliculi are arranged perpendicular to the surface and are interpreted to serve as conduction paths through the cuticular intima for the liquid secreted by the cells to the lumen. Blanc ('89) working on the silk gland cells of *Bombyx mori* concluded that the intima was a chitinous structure composed of very fine parallel threads encircling the lumen at intervals of from 3–4 micra and showing occasional anastomosis. Gilson ('94) confirmed the observations of Blanc. Maziarski ('11) describing the intima, pictures the structure as a thin membrane composed of a great number of filaments passing in spiral lines around the lumen of the canal and joined by fine transverse fibrillæ, thus producing the appearance of a network of threads. These filaments are described as staining deeply with iron hæmatoxylin and having the appearance of rigid fibrils located in the protoplasm of the cell nearest the lumen, and were thought to resemble organs of support but not to serve as passageways for substances elaborated in the cells.

Tanaka ('11) describes the intima as a striated, filamentous-appearing structure of chitinous nature, thickest in the anterior division and thin in the middle region. The filamentous striations appear to have a parallel arrangement except toward the posterior region where anastomosis may occur. This chitinous substance which gives rise to the intima is thought by Tanaka to be secreted in the anterior region and never to undergo ecdysis, but rather is added to in thickness with age. This is (p. 16), he concludes, "a fact which goes a long way in proving the secretion of this chitinous substance throughout the whole larval life."

Yamanouchi ('22) found the intima to be a glassy-like cuticular layer most conspicuously present in the anterior region where it has a thickness twice that in the other parts of the gland. This structure appears to be hyalin in nature, reacting to stains very similarly to sericin but more intensely in the anterior portion of the middle region, this structure is not homogeneous but gives the appearance of containing concentric bands whereby it is possible to distinguish two or three layers of intima. This arrangement in layers disappears anteriorly.

(*b*) *Observation.*—In the gland of *H. cunea* after fixation by the Regaud method, the presence of a clear, hyalin layer of material between lumen and cell-wall is found to be quite generally present in both the middle and anterior regions. (Figs. 7, 8, 9, 12.) This layer is always found to be very closely connected with the cell-wall, and yet to be perfectly distinct from the protoplasm. Oftentimes large vacuoles may be seen in the structure (Fig. 7), but these are always on the edge toward the cell and may be explained as the result of shrinkage at the time of fixation. This vacuolated condition occurs with greater frequency in the posterior portion of the middle region where the layer is thicker and less regular in form.

Posteriorly, in the secreting region, no indication of an intima is found except for the presence of a thin membrane along the inner edge of the cells. The cells usually have an irregular surface on the side toward the lumen, the protoplasm seeming to be in very close connection with the cavity (Fig. 2), but after fixation with Champy, Gatenby, or Benda preparations, this membrane is quite definite (Fig. 1).

Toward the more anterior portion of the middle region, fibrillæ may be seen extending through the clear hyalin substance and around the lumen. In longitudinal sections through the edge of the lumen, these structures appear to extend from the edge of the cell on one side of the lumen to the cell on the other side (Fig. 10), and to take their origin along the margin of the protoplasm. These fibrillæ stain darkly with iron hæmatoxylin and dark red with Altmann's acid fuchsin after fixation by the Champy method. In general, the fibrillæ tend to be arranged in parallel series and some evidence has been found of slight anastomosis between fibrillæ in the more posterior region. After the tube has become narrowed to form the conducting portion, fibrillæ disappear leaving the intima clear and structureless.

(*c*) *Critical.*—The fact that the intima is present in the anterior and middle portions of the gland as a thickened layer seems to indicate that this structure is not connected with active secretion but must serve some function related to conduction of secreted products to the outside. A copious supply of secretion is being poured continually into the reservoir from the posterior region. To strengthen the cell against the resulting pressure and to prevent it from distortion, a thin layer of hyalin material might be conceived to serve as a support. At the point in the tube where it begins to narrow anteriorly, the pressure becomes greater and more support is necessary. That fibrillæ should appear in the transition region between the middle and anterior regions, is, therefore, to be expected. It is quite possible, moreover, that these fibrillæ may be more than merely supporting structures. Their shape and position around the lumen seems to indicate that they may have a contractile function, serving, perhaps, as a simple musculature in pushing the products of secretion into the long conducting tube. It is evident that some regulatory mechanism is necessary to prevent the small tube from becoming clogged by the ever accumulating secretion, and likewise, to regulate the amount of material passing through in order to produce a silk thread of uniform diameter.

4. *The Secretion.*

(*a*) *Historical.*—The morphological appearance of the secreted material has received considerably more attention throughout the literature than the mechanism of the secretion process, and yet little is definitely known about the real nature of the secreted substance. Staining reactions have been used almost exclusively as criteria upon which to base conclusions. Little detailed work has been done upon the actual chemical composition of the silk fiber further than a general analysis of the chemical components, the result of which has been to show that the fiber is made up of two slightly different substances; an outer, acidophilic layer of *sericin*, and a central core of basophilic *fibroin*. The former is quite similar in composition to the latter except that, according to Blanc ('89), it contains more oxygen, dissolves more readily in alkaline solutions and is more granular in appearance.

Tanaka ('11, p. 18–20) has reviewed very completely the results of previous workers and has arranged their views concerning the origin of fibroin and sericin into four principal groups. He then attempts to show that these theories have failed to satisfy all the conditions and proceeds to modify and combine parts of each theory into one more in accord with his own conclusions which he states as follows: "The fibroin is produced by virtue of the physiological function of the gland cells, without reference to any part of the gland tube; the sericin is, in its formation from fibroin, quite indifferent from the physiological function of the cells; on the contrary this transformation goes on merely physico-chemically."

Maziarski ('11) in his enthusiasm for the nuclear origin of secretory products, states that the nucleoli which leave the nucleus become the fibroin and that chromatin, likewise, may be extruded into the protoplasm, there to undergo quite extended changes and finally pass out into the lumen as the sericin or gummy covering of the silk thread. This idea was not entirely new, for Vorhies ('08) had suggested that chromatin material may have a secretory function in addition to serving as the "bearer of heredity."

Nakahara ('11) recognizes only the formation of silk from transformed nucleoli, but Yamanouchi ('22) describes the production of sericin and fibroin in great detail and concludes that fibroin secretion takes place only in the posterior region, but that sericin secretion occurs in the posterior and middle parts of the middle region. The two kinds of secretion appear alternately and, due to the fact that each kind tends to remain separate, two layers are formed, distinguishable by their different staining reactions.

Blanc ('89) has described a third product of secretion, called by him "*mucoidine*," which envelops the silk fiber and stains more intensely with methyl green than either the fibroin or sericin. This substance, he thought, is formed in the anterior portion of the reservoir and may serve the function of a lubricant, thus facilitating the passage of the silk.

(*b*) *Observations.*—From a comparative study of silk gland cells prepared in different fixatives, it is evident that there exist in the lumen, substances which show different types of reactions to stains. After Regaud fixation, the silk thread appears to be perfectly homogeneous throughout its entire extent except in the anterior region of the gland where a slightly darker band may be distinguished around the thread. (Fig. 9.) The secretion found in the posterior and middle portions takes a reddish-blue stain with Altmann's acid fuchsin, methyl green, and in the anterior portion, is decidedly green. The intima takes a bright red stain and appears to be a homogeneous, hyalin substance.

The condition of the secretion product after fixation with Champy, Benda, or Gatenby solutions is considerably different and leads to doubt concerning the identity of the various structures present in the lumen. With iron hæmatoxylin and acid fuchsin, the core of secretion is stained black. In the middle region, the secretion does not fill the lumen as is the case after fixation with Regaud, but between the secretion and the intima, now distinguished as a thin black membrane at the cell border, there is present a compact mass of finely granular substance that takes the acid stain. This substance, however, is stained light green in material fixed by the Champy, and stained by the Altmann method. Usually, the material appears to be homogeneous, but after Benda, and sometimes after Champy fixation, the substance is arranged in thread-like strands that extend from the cell-wall toward the secretion. That this band of substance is separate from the core of secretion is quite evident, for the secretion may be found entirely removed from the substance and be free in the lumen. The substance is never free from the edge of the gland cell. It would seem that either the amount of secretion present is determined by the width of this band which fills the remainder of the lumen or that the size of the band of substance is dependent upon the amount of secretion present, since after fixation, little or no shrinkage apparently occurs and the core of silk is contained in the center of the lumen closely surrounded by a band of this substance. The former hypothesis seems more probable since some regulatory mechanism for producing a thread of uniformly decreasing dimensions must be present.

Material fixed by the Champy, and stained with the Altmann method affords evidence of the presence of two substances in the thread. Secreted substance in the posterior portion of the gland stains light pink on the periphery while the center is either dark pink or green. This condition is not seen as sections are selected in the more anterior part of the gland. After fixation of the gland in Bouin, the silk thread exhibits two distinct staining reactions. The core stains a clear pink and the periphery forms a black cortex around this core. This reaction of the secreted substance is consistent throughout the tube.

In silk glands of *D. virginica* fixed in Bouin and stained in iron hæmatoxylin and acid fuchsin, the silk thread has a central core of clear pink substance surrounded by a black peripheral cortex. A wide region filled with a reticulum or meshwork of fine pink granules surrounds the thread. Bordering this region and extending to the inner cell-wall, a wide compact band of fine granular substance arranged in concentric rings occurs. Throughout this band, numerous large drops of black substance appear which probably represent secretion material. By comparison of the condition just described, with that found in *H. cunea*, and also with conditions described by Yamanouchi, it appears that some difference exists between the type of gland which secretes continuously and that which secretes at definite periods only. The presence of two diverse staining reactions in the thread seems to indicate that two substances are consistently coexistent in the two different types of silk gland cell, but the exact relationship between these two substances has not been determined with certainty.

(*c*) *Critical.*—The question as to the identity of the substances within the lumen has presented a problem which cannot be definitely settled without further study both of preserved material from a variety of caterpillars and also of living material. Observation of the material at hand seems to show that in the posterior portion of the gland, the secreted substance is usually homogeneous, and this is likewise true in the middle region. But some modification of the secreted substance takes place in the anterior conducting region which gives the silk thread the appearance of being composed of two substances.

It is evident that even in forms which secrete silk during the entire larval life, the process is not a continuous one, for often within the lumen of the silk gland, the end of one thread can be seen and, close upon the posterior part of this thread, but separated from it by the substance filling the lumen, the end of another discrete cylinder of secretion begins (Fig. 7).

The substance which fills all available space in the lumen not occupied by silk secretion, may well be similar in its properties to mucus, lubricating the tube for the passage of the silk fiber. No origin for this substance has been found. It does not appear to be secreted by the cells of the gland, nor does it seem to be a dislodged part of the bodies of the cells.

Discussion.

1. *Relation of the Nucleus to Secretion.*

The nucleus and cytoplasm of the cells of the silk gland are closely associated in the production of the silk secretion. By many investigators, the nucleus has been considered the center of secretory activity in that the nucleoli migrate from the nucleus into the cytoplasm and there undergo modification. Study of the silk gland cells of *H. cunea* has given further indication of nuclear participation in silk production through the presence of nucleoloid bodies. These nucleoloid bodies do not themselves pass into the cytoplasm but appear to constrict pieces from their periphery that increase in size within the nucleus, finally passing through the nuclear wall into the cytoplasm where further modification of their substance occurs.

2. *Relation of Mitochondria to Secretion.*

Mitochondria having a characteristic arrangement in the cytoplasm of the secreting region are present in great numbers. It is quite possible that they take some part during the transitory stage when the secretion droplets are present in the cytoplasm, in modifying the material and transmitting it to the lumen. Mitochondria may act as catalysts, as suggested by Emberger ('25) or they may take a more direct part in secretion, but their presence and orientation toward the lumen seem to indicate that they are not passive cytoplasmic inclusions. Mitochondria are present throughout the extent of the gland but in the middle and anterior portions, they are shorter than in the secretory portion and show little definite orientation. In these portions, they may aid in the process of conduction and in general metabolic activity.

3. *The Nature of the Secretion.*

The column of silk secretion is apparently composed of two slightly different substances as evidenced by diverse staining reactions, but further study is necessary before the exact origin and nature of each is determined.

Since in the posterior region, the silk thread appears to be a homogeneous cylinder when preserved in several different fixatives, it would seem that there is but one kind of substance secreted and that this substance undergoes partial modification, possibly oxidation, during its progress down the tube.

A substance similar to mucous appears in the lumen as an envelop surrounding the silk column, but no account of its origin can be given. In life, it is evidently a fluid substance because it is found to be present wherever a break in the continuity of the fiber occurs. The distinction between this substance that is finely granular in appearance after certain fixations and the clear hyalin substance present in the same relative position, following fixation in Regaud's solution, cannot be determined. The latter substance has been considered as composing the intima but the former cannot be so accounted for.

SUMMARY.

1. In the silk gland of *Hyphantria cunea*, three regions are distinguishable; a posterior, or secreting region; a middle region, or reservoir; and an anterior, or conducting region.

2. The nuclei of silk gland cells are greatly branched in the posterior region, but tend to be more flattened and less branched in the more anterior regions.

3. Three types of bodies are found to be present in each nucleus; the small basophilic granules, or chromatin; the intermediate acidophilic bodies generally termed nucleoli, but here called nuclear bodies; the relatively large, spherical body containing vacuoles and corresponding to a true nucleolus, hence called a "nucleoloid body."

4. The nucleoloid bodies appear to give rise to the nuclear bodies which pass out of the nucleus into the cytoplasm.

5. Cytoplasmic granules exhibit the same staining reactions as nuclear bodies and are approximately the same in size when they occur in the region of the nucleus.

6. Mitochondria are present in all parts of the gland, but only in the posterior region do they show definite orientation.

7. Two membranes are present along the course of the gland, and external tunica propria, and an internal tunica intima.

8. Two substances regularly appear to constitute the silk thread in the anterior region. This condition is found to continue more posteriorly after fixation with the Champy, Gatenby, Benda, and Bouin methods.

9. A substance similar to mucous surrounds the silk thread in the middle region but does not extend into the anterior portion. The origin of this substance has not been determined.

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EXPLANATION OF PLATES.

PLATE I.

FIG. *I*. Diagrammatic outline of silk gland of *H. cunea*. Anterior, middle, and posterior regions of gland numbered 1, 2, and 3 respectively. Letters correspond to sections taken at these levels.

FIGS. *A–F*. Camera lucida outlines of sections from the three regions of a tubule fixed by the Regaud method. Cross sections cut 4 micra in thickness; magnification, oc. 10 ×, obj. 4 mm.

Note: Stippling represents cytoplasm; solid lines represent secretion; broken ines represent intima.

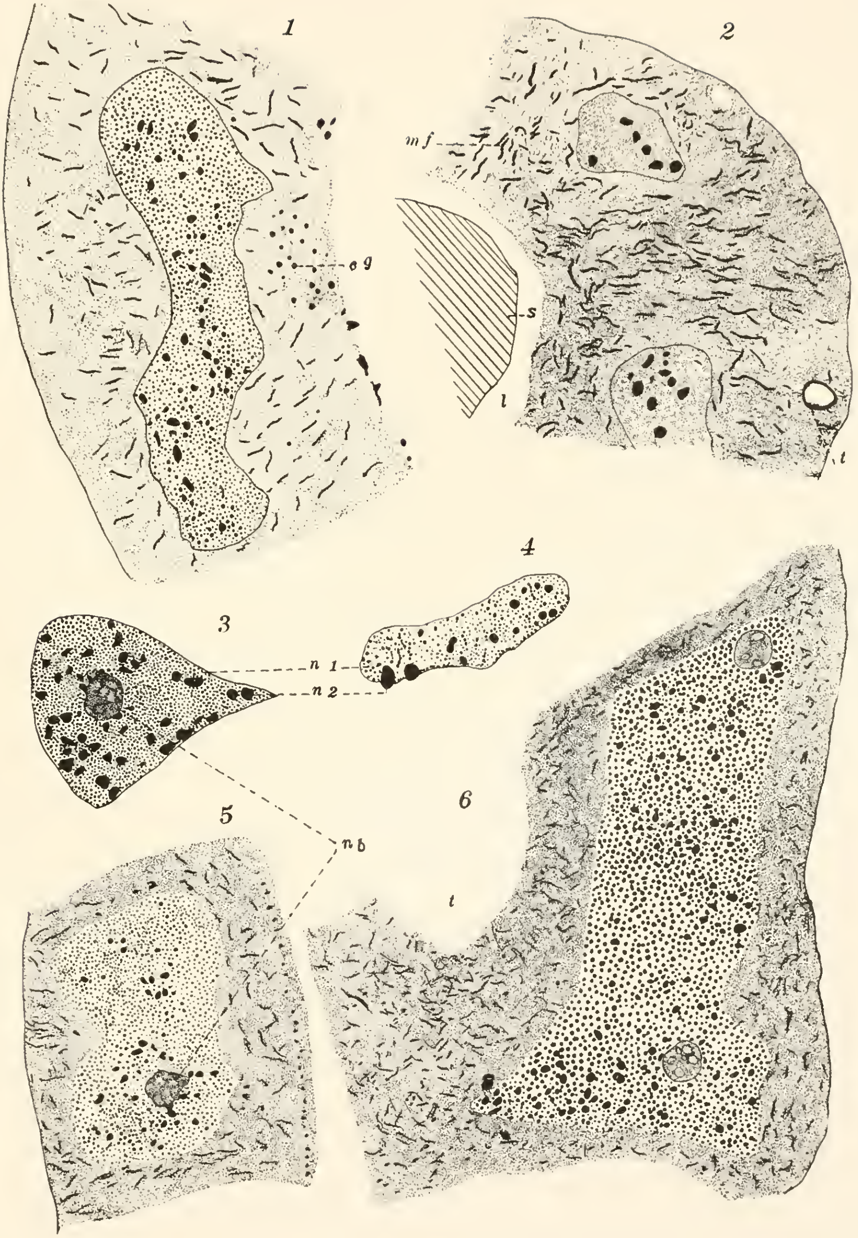
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PLATE II.

All figures made from sections cut 4 micra in thickness, and drawn with camera lucida, magnification, oc. 12.5 ×, obj. 1.8 mm., oil immersion.

FIG. 1. Section of cell from posterior region of tubule fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Cross section. Cytoplasmic granules appear to be grouping toward lumen.

FIG. 2. Section of cell from secreting region of tubule fixed by Regaud method and stained with acid fuchsin, methyl green. Cross section. Mitochondria show definite orientation toward lumen.

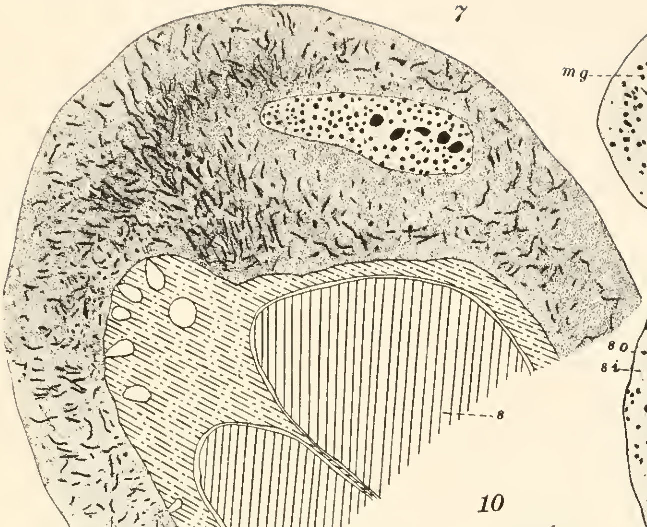
FIG. 3. A section of a nucleus from material fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. The nucleoloid body is surrounded by nuclear bodies that are smaller than those toward the nuclear wall.

FIG. 4. Section of nucleus from material fixed by the Gatenby method and stained with iron hæmatoxylin, acid fuchsin. Two nuclear bodies are in the process of passing through the nuclear wall into the cytoplasm.

FIG. 5. Longitudinal section of cell from posterior region of gland fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Nucleoloid body in process of constricting pieces from periphery.

FIG. 6. Longitudinal section of cell showing two nucleoloid bodies present in nucleus. Regaud fixation.

Symbols: *cg.* cytoplasmic granules; *l.* lumen; *mf.* mitochondrial filaments; *n1.* chromatin; *n2.* nuclear bodies; *nb.* nucleoloid body; *s.* secretion.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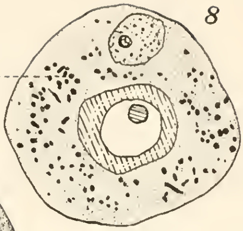
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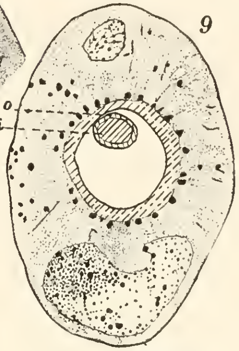
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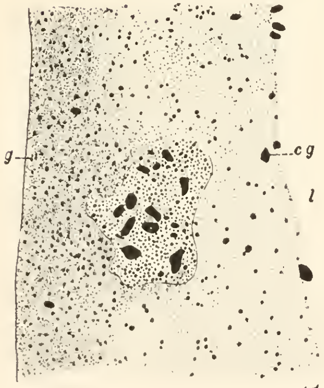
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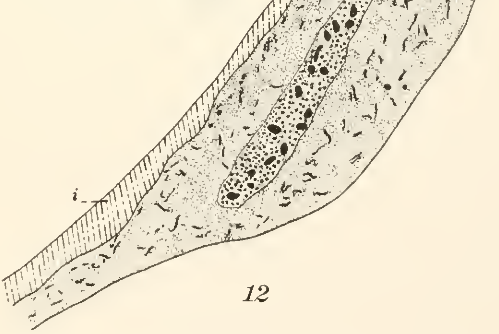
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PLATE III.

FIG. 7. Section of cell taken from posterior part of middle region of gland fixed by the Regaud method and stained with acid fuchsin, methyl green. Cross section. Hyalin layer appears irregular and contains vacuoles along the border toward cell. Mitochondria slightly shorter. Two columns of secretion chance to be present at this level.

FIG. 8. Cross section of tubule from anterior conducting region of gland fixed by the Regaud method and stained with acid fuchsin, methyl green. Mitochondria present as granules. Intima definite.

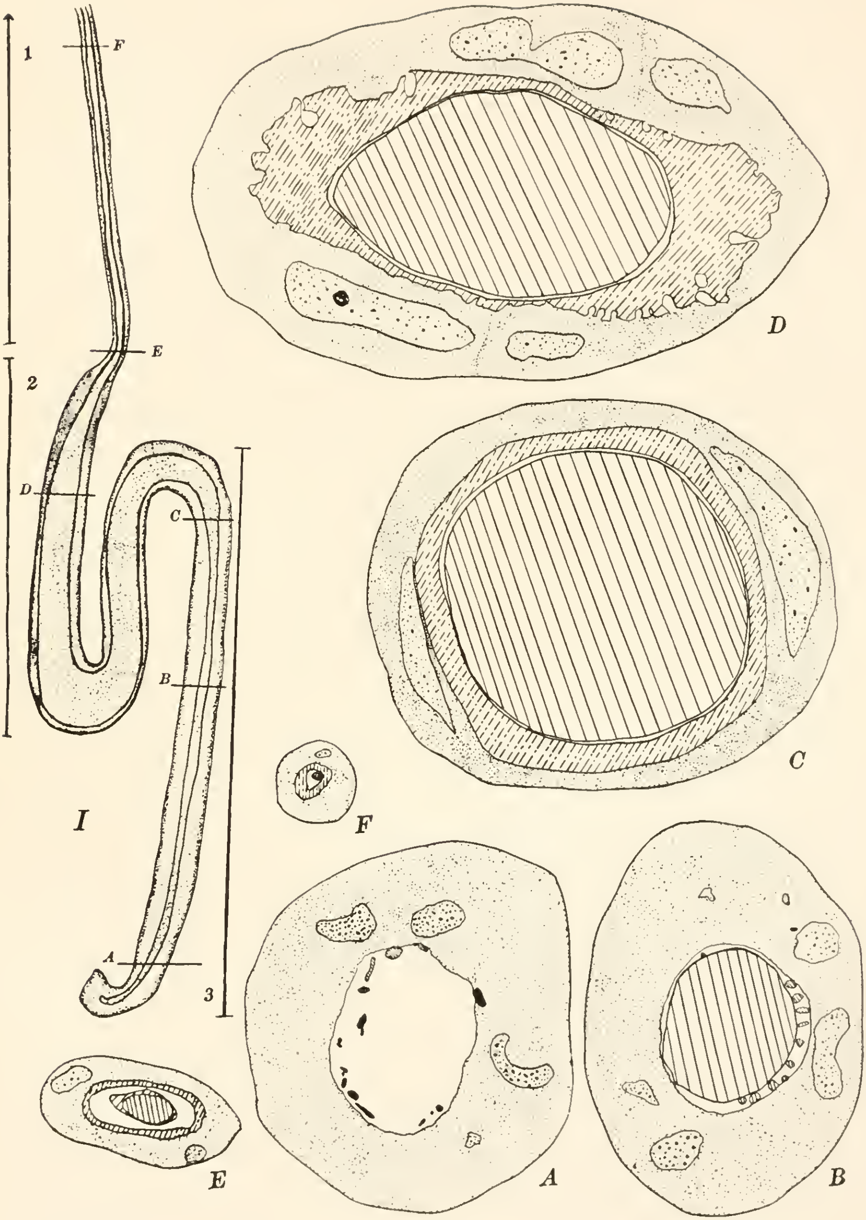
FIG. 9. Cross section from anterior conducting region of tubule fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Mitochondria appear as granules. Intima well defined, two types of secretion present.

FIG. 10. Longitudinal section of tubule fixed by the Champy method and stained with iron hæmatoxylin, acid fuchsin. Section taken from anterior part of the middle region at edge of lumen. Fibers appear extending from the edge of one cell to the edge of the cell opposite.

FIG. 11. Longitudinal section of cell from secreting region of tubule fixed by the Mann-Kopsch method, unstained. Numerous fine black granules fill cytoplasm and tend to concentrate toward the outer cell boundary.

FIG. 12. Cross section of cell from middle region of tubule fixed by the Regaud method, stained with acid fuchsin, methyl green. Mitochondria are shorter and less definitely orientated. Clear hyalin intima present along the edge toward lumen.

Symbols: f., fibers; *g.*, granules; *mg.*, mitochondrial granules; *p.*, tunica propria; *si.*, inner layer of secretion; *so.*, outer layer of secretion.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ELIZABETH KINNEY.

THE LINKAGE DISTURBANCE INVOLVED IN THE CHROMOSOME TRANSLOCATION I. OF DROSOPHILA, AND ITS PROBABLE SIGNIFICANCE.

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The chromosomal abnormality in *Drosophila* known as "translocation I." was found by Bridges in 1917, and was first referred to by Morgan (1919); a preliminary account of it was given by Bridges in 1923. The case is a very remarkable one, unique in the literature to date, and it raises certain new problems concerning the arrangement which genes may have in a chromosome as well as concerning the changes in arrangement which they may undergo. The abnormality arose by a portion of the right hand end of chromosome II. breaking off and becoming attached near the middle of the right half of chromosome III. Bridges' data showed the size of the transposed piece and its approximate point of attachment on the third chromosome. It was known that the break in chromosome II. was between arc (ar) and plexus (pl), making the fragment about 8 units long, and that the transposed piece was attached somewhere between ebony (e) and rough (ro) in chromosome III. The deficient second chromosome was known as "Pale," from its effect on eosin-eyed flies, and the deficiency was lethal unless accompanied by translocation.

In the hope of getting further light on the chromosome change involved, the writer undertook to test more extensively the third chromosome linkage values in flies which carried the translocation. I wish to take this opportunity to express my indebtedness to Dr. H. J. Muller, who suggested the work, and whose aid and suggestions have made it possible. In these experiments the homozygous as well as the heterozygous condition of translocation was studied, and all of the left hand end of Chromosome III. was under genetic observation. Bridges had already shown that crossing over was greatly reduced in the region close to the locus of translocation; but his data included only flies heterozygous for the transposed piece. The comparison of the homozygous and the heterozygous conditions with each other is of especial interest because of its bearing on the problem of the mode of attachment of the second chromosome fragment, as will be shown later.

The third chromosome mutant genes used in the experiments are, in their order from left to right along the chromosome: roughoid (ru), hairy (h), scarlet (st), pink (p), spineless (ss), delta (Δ), hairless (H), ebony (e), rough (ro), and claret (ca). Their locations are shown in the map of Fig. 1. Translocation is represented by Tr, and the deficient second chromosome by Pl. Curly (Cy) is a second chromosome gene used in balancing stocks. The linkage in flies homozygous for Tr was tested in two experiments. The summarized results for these crosses are given below, in Tables I. and II.

TABLE I.

$$\frac{Cy}{Pl}\ \frac{\Delta\ H\ e\ Tr}{ru\ h\ st\ p\ ss\qquad Tr}$$ ♀ × ru h st p ss e ♂.

Regional Crossover Frequencies.						Total.
ru–h.	h–st.	st–p.	p–ss.	ss–Δ.	Δ–H.	
285	202	45	86	61	10	1,097
26.0%	18.4%	4.1%	7.8%	5.6%	.9%	

TABLE II.

$$\frac{Cy}{Pl}\ \frac{\Delta\ H\ e\ Tr}{Tr\ ro\ ca}$$ ♀ × e ro ca ♂.

Regional Crossover Frequencies.				Total.
Δ–H.	H–e.	e–ro.	ro–ca.	
8	13	27	16	463
1.7%	2.8%	5.9%	3.5%	

These figures show a remarkable drop below normal in the crossover values as the locus of translocation is approached. This may be seen by comparing their map values (see Fig. 1, *b* and *c*) with the normal third chromosome map (Fig. 1, *a*). The map length of the left-hand third will be seen to be slightly increased, but the genes between pink and claret are crowded much closer together. There is an apparent lengthening of the

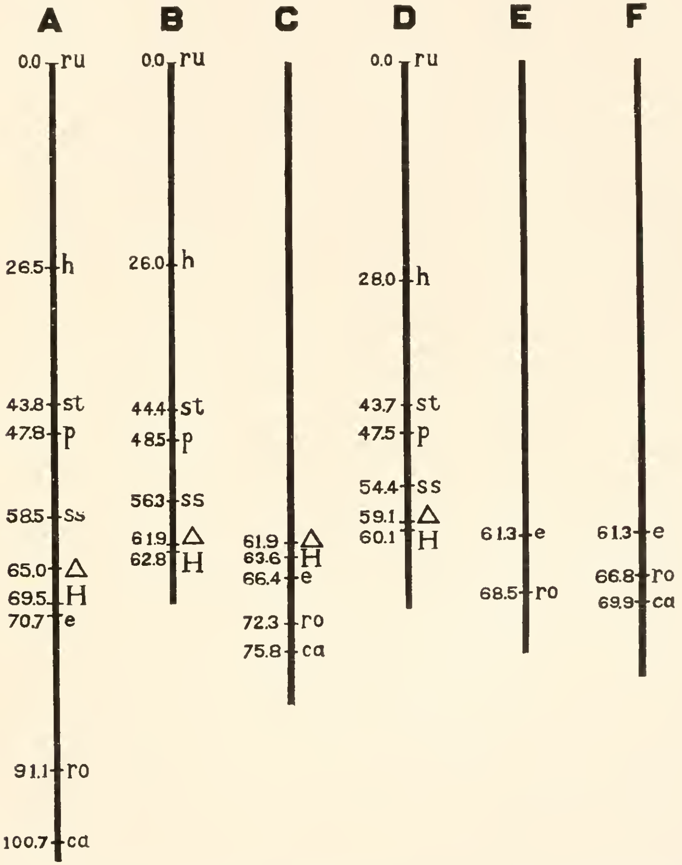
A
B
C
D
E
F
0.0 ru
26.5 h
43.8 st
47.8 p
58.5 ss
65.0 Δ
69.5 H
70.7 e
91.1 ro
100.7 ca
0.0 ru
26.0 h
44.4 st
48.5 p
56.3 ss
61.9 Δ
62.8 H
61.9 Δ
63.6 H
66.4 e
72.3 ro
75.8 ca
0.0 ru
28.0 h
43.7 st
47.5 p
54.4 ss
59.1 Δ
60.1 H
61.3 e
68.5 ro
61.3 e
66.8 ro
69.9 ca

FIG. 1. (*a*) The normal third chromosome map. (*b*) and (*c*) Maps of third chromosome homozygous for translocation. (*d*), (*e*) and (*f*) Maps of third chromosome heterozygous for translocation.

distance between hairless and ebony, but this distance is so short, and the probable error so large, that the increase is probably not significant. Between ebony and rough, the region where the second chromosome fragment is attached, the per cent. of crossing over is less than one third of the normal value. These relations are shown in Fig. 2, *a*, which shows the ratios of the values obtained in these experiments to the standard values of each of the major regions studied.

At the same time that these crosses were being made, the linkage in flies heterozygous for the abnormality was being tested. The same stocks were used in making up the crosses, and the characters used were introduced into the cross in as near the same way as possible. Both the homozygous and heterozygous lines were kept under the same conditions, so that any possible variations in viability would affect both lines alike and allow us to draw valid comparisons between them. As will be seen in Tables III. and IV., the results were almost identical with those of the first tests.

Table III.

$$\frac{}{\text{Pl}}\;\frac{\Delta\ \text{H e Tr}}{\text{ru h st p ss}\qquad\text{e}}\ \text{♀} \times \text{ru h st p ss e}\ \text{♂}.$$

Regional Crossover Frequencies.						Total.
ru–h.	h–st.	st–p.	p–ss.	ss–Δ.	Δ–H.	
162	91	22	40	27	6	578
28.0%	15.7%	3.8%	6.9%	4.7%	1.0%	

Table IV.

$$\frac{}{\text{Pl}}\;\frac{\text{ru h st p ss}\qquad\text{Tr}}{\text{p ss e}\qquad\text{ro}}\ \text{♀} \times \text{p ss e ro}\ \text{♂}.$$

Crossovers.		Total Flies.
(ru h st) p ss e	(ru h st) p ss ro	
42	41	
Total Crossovers	83	1,155
7.2%		

In Table V. are results for a test involving the ebony-rough and rough-claret distances. Only those flies which did not show delta hairless could be used in computing crossover values in this experiment. This cross also gave data on the exact location of translocation, as will be discussed later.

Table V.

$$\frac{Cy}{}\,\frac{ru\ h\ st\ p\ ss\quad Tr}{e\quad ro\ ca}\ \text{♀} \times \frac{Cy}{Pl}\,\frac{\Delta\ H\ e\ Tr}{e\quad ro\ ca}\ \text{♂}.$$

Regional Crossover Frequencies.		Total Utilizable Count.
e–ro.	ro–ca.	
50	28	902
5.5%	3.1%	

The results given in the last three tables are summed up in the chromosome maps shown in Fig. 1, *d*, *e*, and *f*, and Fig. 2, *b* shows the ratios of these values to the standard values. It will be seen on comparing these results with those based on the homozygous Tr flies that there is no noticeable difference in the crossover effects produced by the homozygous and by the heterozygous conditions.

The chief significance of the results probably lies in their bearing on the mode of attachment of the translocation. There are two conceivable ways in which the translocated piece might be attached between ebony and rough: it might be interpolated within this length, thus causing a prolongation of the thread between ebony and rough, or it might be attached somehow to the side of the original thread, thus resulting in a configuration of genes and of chromatin different from what had previously been regarded as normal, and not entirely in one line. The present results clearly disprove the first alternative, for if it were true, crossing over between ebony and rough should be increased when the flies were homozygous for the interpolated fragment. We must therefore conclude that the chromosome carrying translocation is branched or doubled in the region where the latter is attached.

The second point of significance is that the presence of translocation does not reduce crossing over throughout the chromosome. Beginning at the left-hand end of the chromosome, we find that the number of crossovers is practically normal until we reach the locus of pink, one third of the distance along the chromosome. From this point on, the map distance is markedly shortened until the locus of translocation is reached. In this interval, between ebony and rough, the per cent. of crossing over is less than one third that of the normal. Between rough and claret the amount of crossing over rises again, approaching half the normal value. The number of flies involved was not large enough to yield reliable data on the coincidences, but as far as they went they agreed well with the normal.

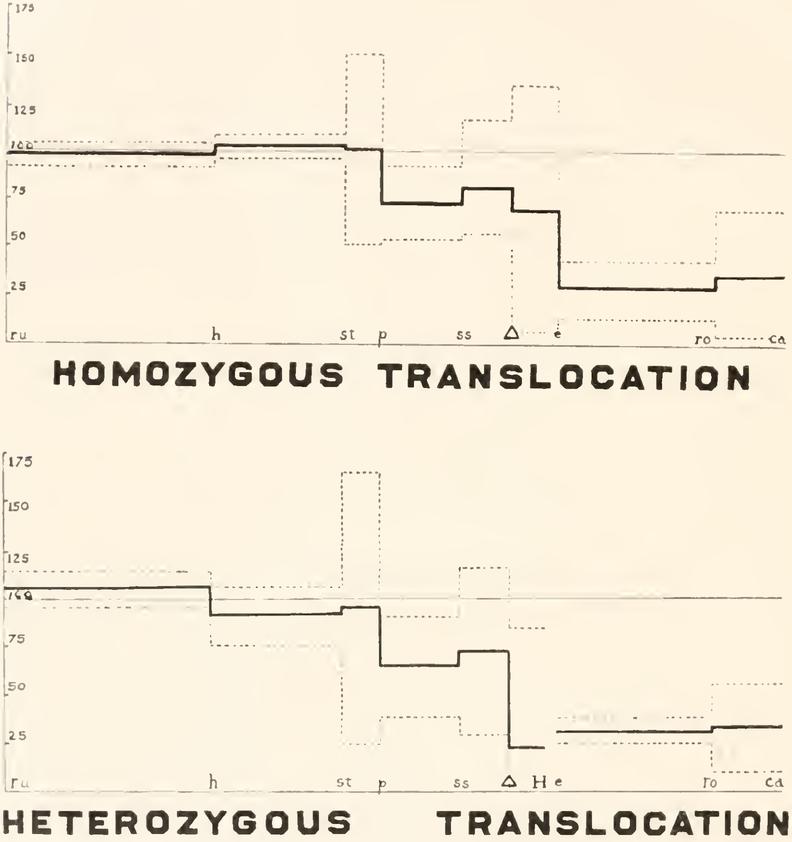

FIG. 2. Chart showing the ratios (in percentages) of the observed crossover values to the standard. The abscissa represents the length of the III. chromosome from roughoid to claret; the ordinate represents the percentage which the observed crossovers form of the standard value in each region. The heavy line represents the percentage observed; the dotted lines mark the limits of the probable error of this percentage, calculated for each interval.

If crossing over takes place at a stage in synapsis in which the chromosomal threads are twisted about each other, we can readily see why the presence of the attached fragment in either one or both of the third chromosomes should produce the effects that it does. Either by stiffening the threads so that they could not twist as tightly as in the rest of the chromosome, or by an actual interference with the loops, the transposed piece should tend to prevent the close association between the homologous chromosomes necessary for that interchange of genes which constitutes crossing over. This interference would produce its maximum effect at the point of attachment, and the effect would decrease to either side. This gradual decrease in the interference is well shown to the left of ebony. The locus of translocation is too close to the right-hand end of the chromosome to show any marked diminution of its effect toward that side. The rough-claret crossovers are, however, nearer to the normal percentage than those in the ebony-rough interval.

In the cross shown in Table V., 36 crossovers between ebony and rough were observed in which it was possible to follow the behavior of the translocation. The results are shown below.

Table VI.

$$\frac{\text{Cy}}{\ }\ \frac{\text{ru h st p ss}\quad\text{Tr}}{\text{e}\quad\text{ro ca}}\ ♀ \times \frac{\text{Cy}}{\text{Pl}}\ \frac{\Delta\ \text{H e Tr}}{\text{e}\quad\text{ro ca}}\ ♂.$$

Crossovers between (e) and (Tr)	Crossovers between (Tr) and (ro)
$\frac{\ }{\text{Pl}}\ \frac{\text{e Tr}}{\text{e}\quad\text{ro ca}}$	$\frac{\ }{\text{Pl}}\ \frac{\text{(ru h st p ss)}\quad\text{Tr ro (ca)}}{\text{e}\quad\text{ro ca}}$
11	25
36	

Translocation is, according to these figures, located at approximately 11/36 of the distance between ebony and rough, or at 76.9 on the chromosome map. The data given in Bridges and Morgan's monograph on the III. chromosome characters also show translocation to be between ebony and rough, but their figures show the locus of translocation to be closer to rough than to ebony. Also, the amount of crossing over in the ebony-rough interval is even lower than in the experiments here reported. These differences may be due to a differential viability in the two cases; there may also be differences in genes influencing crossing over, but such differences in ratios do not affect the principal conclusion of this paper. The important point is that the effect of the translocation is the same whether it is heterozygous or homozygous.

SUMMARY.

1. The chromosomal abnormality in *Drosophila*, known as translocation I., is located at approximately 76.9 on the third chromosome.

2. The presence of translocation does not affect the crossover values for the first 45 units in the left-hand end of the chromosome. To the right of this point, the per cent. of crossing over progressively decreases as the locus of translocation is approached, reaching its minimum of less than one third normal value in the region between ebony and rough.

3. The same results are obtained no matter whether the translocation is homozygous or heterozygous.

4. The fact that the results are the same in both heterozygous and homozygous flies shows that the attached fragment is not interpolated in the third chromosome, but is attached to its side. The resulting chromosome is thus of a type which has hitherto not been reported: that is, one in which the genes are not in a single linear series.

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THE CHROMOSOMES OF THE CHICK SOMA.

ROBERT T. HANCE,

THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH AND THE ZOÖLOGICAL LABORATORY, UNIVERSITY OF PENNSYLVANIA.[1]

The accumulated evidence in favor of the chromosomes being in some way concerned with the determination and control of the somatic characters has become so impressive as to be almost unassailable despite the possible objection of a few that the last link in the chain (a demonstration of the actual activity of these nuclear bodies as hereditary character bearers), due to the deficiencies in our knowledge of cell chemistry, has yet to be forged. This latter point is one about which geneticists and cytologists are, at present, not greatly concerned and conclusive data upon the physiological behavior of the chromosomes does not seem at all imminent. While information is gradually accumulating in preparation for this final analysis much may still be accomplished by purely morphological studies with tentative interpretations of physiological activity based on these observations. A recent study of the chromosomes associated with sex in the chick (5) involved extensive observations on the chromosomes of dividing somatic cells, the results of which are recorded below.

OBSERVATIONS.

The technique of preparation (3), of counting and measuring the chromosomes, the amount and source of the material (5) have all been recorded and need not be repeated here. The chromosomes of over 150 cells have been drawn and studied. As the nervous system is the most actively growing of all the regions of the body the majority of the cells studied have naturally been found in some part of it. Cells with clear polar views of metaphase chromosomes have been found in the brain, neural tube, optic cup, auditory vesicle, connective tissue, heart muscle, blood, amnion, gonads and in tissue cultures of muscles.

---

[1] The observations recorded in this paper were made principally at the University of Pennsylvania during the writer's tenure of a National Research Council Fellowship.

The Chromosome Number.—The difficulties involved in determining the exact number of chromosomes in the chick have been described in detail (5). Briefly, the trouble encountered may be said to be due, first to the extreme smallness of the shortest chromosomes of the complex making observation at times uncertain and second, to the apparent failure of the component granules of the smaller chromosomes to unite or at least to clearly indicate their proper relations to each other until late metaphase if indeed it really and always happens then.

The average number of chromosomes in the soma of the chick, based on 78 counts, is 33. I think that this is perhaps lower than the actual number which the most satisfactory counts indicate to be about 35 or 36. This variation in the chick is apparently due to a failure of the chromomeres or parts of the smaller chromosomes to unite rather than to fragmentation as in the case of the pig (1). This opinion is based upon the observations in the chick of larger numbers of distinct chromatin bodies (with occasional visible connecting threads between them) in the prophase than could be found in cells in later stages of mitosis whereas in the pig a reduction in the chromosome number as the metaphase was neared did not occur. The smaller chromosomes of the chick are the only ones concerned while in the pig the long ones are the ones that become broken up.

The Chromosome Form.—All of the longer chromosomes (about 12 in number) of the somatic cells are in the form of J's while the shorter ones are rods. Their structure and size relations (5) are alike in all the tissues studied including tissue cultures (4). The largest chromosome (chromosome pair in the male) of the complex has been shown to be the one associated with sex and is found in all somatic cells as clearly as in the cells of the gonads (5), Figs. 1 to 9. The female embryonic cells are heterozygous for this chromosome while the male cells are homozygous. No differences in the chromosomes or their behavior have been noted between any of the body tissues or in comparison with those of the embryonic germ cells.

Discussion.

The morphological data on the behavior of the chromosomes in the developing embryo, although admittedly scant, has so far given no clue to the manner in which the chromosomes may contribute their potentialities to the growing organism. Before a study of somatic chromosomes had been made it seemed reasonable to expect to find the various highly differentiated cells of the body with chromosome numbers, morphology or behavior at variance both with those found in other tissues and with the specific number and general characteristics found in the gonads. This has been found not to be the case in at least three forms, the pig (1), the evening primrose (2) and the chick (5). There was some fragmentation of the somatic chromosomes in the first two forms but it was shown that none of the chromatin was lost. Furthermore this fragmentation was neither specific for any particular tissue nor constant in amount. In general the chromosome situation in the soma seems to be entirely similar to that found in the unreduced gonad cells. This is a matter difficult to understand in the present state of our information. Of all the characteristics that must be controlled or borne by or at least associated with the chromosomes (as shown by the studies on *Drosophila*) few have any chance for expression in the majority of the somatic cells and tissues and must therefore be inhibited in one way or another. The cells of the lower forms of animal life largely retain the germ-like power of reproducing the portion lost or even an entire organism following injury. This power is perhaps even more marked in plants. As differentiation becomes more extreme in the animal kingdom the ability to regenerate a part or a whole animal from cells already specialized becomes less and less until in the highest types of animals somatic cells are usually able to produce only somatic cells like themselves. Yet the chromosomes in these highly specialized cells have, in the examples studied, been found to be entirely similar to those in the germ cells containing the possibilities for a complex animal or plant. This may suggest that the chromosomes are functionless in the differentiated Soma. Or it may be that having contributed their share in the production of the specialized tissue are thereafter inactive as far as are concerned the general somatic attributes or determiners with which originally they must have been equipped. If the latter interpretation is correct the perpetuation of the complete mitotic mechanism in the soma as it exists in the reproductive organs may seem, as far as any need for an exact division of the genes is concerned, somewhat unnecessary, to be classed possibly with vestigial organs and having no more significance. In view of the great delicacy of the mechanism that exists in all cells for accurately dividing the chromatin the last suggestion does not seem impressive. But as far as our morphological data on the behavior of somatic chromosomes go, together with the behavior of the soma in growth and regeneration, the above suggestion is at least a possibility to be considered in our attempt to get at the physiology of development and genetics.

SUMMARY.

1. The chromosome number in the somatic cells of the chick is about 35 or 36.

2. No characteristic differences between the number, the sizes, the morphology or the behavior of the chromosomes in comparison either with each other or with the cells of the gonads have been noted.

3. In view of the entire similarity of the somatic and germinal mitotic behavior and in consideration of the complete inability of highly specialized cells to regenerate other than cells similar to themselves it is tentatively suggested as a basis for future discussion that the somatic chromosomes, as far at least as their genetic function is concerned, have either become functionless or their cytoplasmic environment is incapable of reacting to the possibilities presumably carried by them.

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DESCRIPTION OF PLATE I.

All figures were drawn at table level with a Zeiss 1.5 mm. apochromat and a 15 × Orthoscopic ocular. They are reproduced at the size originally drawn. All are polar views of metaphase plates in chick embryonic cells with the exception of Fig. 5.

FIG. 1. From optic cup. 35 chromosomes.

FIG. 2. From amnion. 30 chromosomes.

FIG. 3. From a male gonad. 39 chromosomes.

FIG. 4. From neural tube. 32 chromosomes.

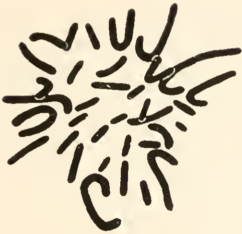
FIG. 5. Late prophase in connective tissue cell grown in a tissue culture. 38 chromosomes.

FIG. 6. From heart muscle. 33 chromosomes.

FIG. 7. From brain. 35 chromosomes.

FIG. 8. From female gonad. 36 chromosomes.

FIG. 9. From neural tube. 35 chromosomes.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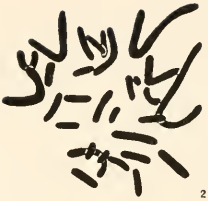
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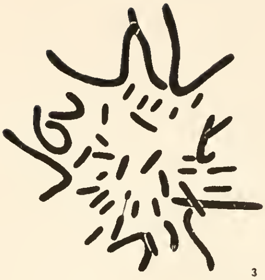
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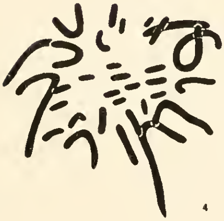
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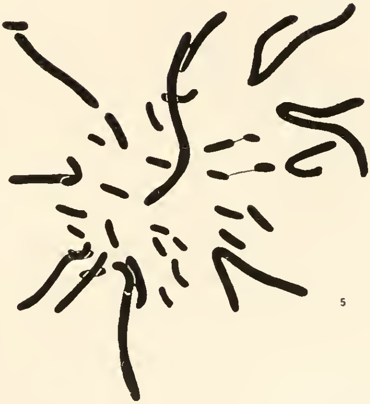
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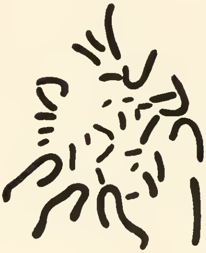
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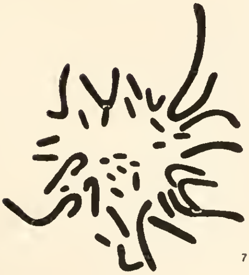
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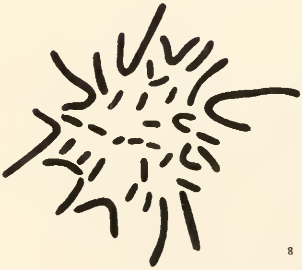
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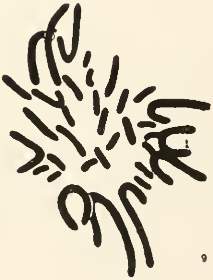
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